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Wastewater sludge treatment by anaerobic digestion with recuperative thickening

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CERTIFICATION

I, Shufan Yang, hereby declare that this thesis, submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy, to the School of Civil, Mining and Environmental Engineering, Faculty of Engineering and Informatics Science, University of Wollongong is wholly my own work unless otherwise referred or acknowledged. The document has not been submitted for qualification at any other academic institution.

Shufan Yang

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Brief Description of Research

This research studied anaerobic digestion with recuperative thickening for energy recovery, biosolids reduction and contaminants' removal.

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Abstract

In most wastewater treatment plants (WWTPs), sewage sludge is treated by anaerobic digestion to convert the biodegradable organic content into biogas, which is a sustainable energy resource. Due to the increasing pressure on wastewater treatment infrastructure caused by ongoing urbanization and population growth, it is necessary to increase the treatment capacity of an existing WWTP meet the increasing wastewater inflow. Recuperative thickening is one of the approaches which can decouple sludge retention time (SRT) from hydraulic retention time (HRT). This project aims to study the effect of recuperative thickening on the anaerobic digestion performance, trace organic contaminants (TrOCs) removal, biosolids reduction and microbial community structure shift.

This project consists of four major studies. The first study focused on the occurrence of TrOCs in wastewater sludge and their removals by anaerobic digestion. In this study, 18 TrOCs were detected in primary sludge. Some of these TrOCs (e.g. paracetamol, caffeine, and ibuprofen) were found at very high concentration in the aqueous phase probably due to their widespread consumption in society. The overall removal of TrOCs by anaerobic digestion was governed by their molecular structure. While an increase in SRT of the digester resulted in an increase in basic biological performance, the impact of SRT on TrOC removal was negligible.

The second study aimed to evaluate the effect of recuperative thickening on the anaerobic digester performance. Recuperative thickening led to an increase in biogas production and system stability due to increment in SRT, and enrichment of methanogens in the digesters. Recuperative thickening also improved sludge dewaterability and reduced odour compounds in biosolids. However, recuperative thickening barely enhanced the organic matter destruction at a sufficiently high baseline SRT value. Thus, recuperative thickening would be a viable technique to improve the performance of digesters with inadequate SRT or issues with system stability.

Shearing was studied in the third part of research in terms of biogas production, microbial community structure and TrOCs' fates. Medium shearing improved biogas production, while high or excessive shearing reduced the biological performance. Microbial analysis showed that medium shearing increased the evenness and diversity of the microbial community of digestate. In agreement with the observed decrease in biogas production, the abundance of hydrolysis and

acetogenesis related microbes decreased due to high shearing. On the other hand, 17 TrOCs were detected in all sewage sludge samples. Hydrophilic and readily-biodegradable TrOCs were well removed under all conditions. Carbamazepine, gemfibrozil, and diuron were only biodegraded at high shearing. It is possible that shearing can facilitate the circulation of TrOCs between aqueous and solid phases, thus, enhancing the biodegradation of some TrOCs.

The fourth study combined two approaches, thermal pre-treatment and recuperative thickening, to anaerobic digester in order to achieve better energy recovery, solid reduction and TrOC removals. Thermal pre-treatment (150 °C, 30 min) for primary sludge enhanced the biogas production by 15%; however, thermal pre-treatment or recuperative thickening barely affected the solid and tCOD reduction. Again, 16 trace organic contaminants were constantly detected, and compounds like caffeine, sulfamethoxazole, trimethoprim and paracetamol were highly degraded during anaerobic digestion. Thermal pre-treatment and recuperative thickening were effective to improve the biodegradation of a few hydrophobic compounds like clozapine, triclosan and triclocarban.

Keywords: anaerobic digestion, recuperative thickening, biogas production, TrOC, shearing, thermal pre-treatment, microbial community structure

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List of Abbreviations

DAFT	Dissolved air flotation thickening
EPS	Extracellular polymeric substances
HRT	Hydraulic retention time
MBR	Membrane bioreactor
OLR	Organic loading rate
RT	Recuperative thickening
SRT	Sludge retention time
SVI	Sludge volume index
sCOD	Soluble chemical oxygen demand
tCOD	Total chemical oxygen demand
TS	Total solid
TP	Thermal pre-treatment
TrOC	Trace organic contaminant
VS	Volatile solid
WWTP	Wastewater treatment plant

List of Symbols

logD	Distribution coefficient at logarithmic scale (The logarithm of a ratio of the sum of the experimentally measured concentrations of the solute's various forms in one solvent, to the sum of such concentrations of its forms in the other solvent.)
pKa	Acid dissociation constant at logarithmic scale

Chapter 1 Introduction

1.1 Background

1.1.1 Brief introduction of wastewater treatment processes

Wastewater or sewage treatment, is an essential part of waste management of municipal authorities. Wastewater treatment plants (WWTPs) generally conduct three major steps for wastewater, namely primary, secondary and tertiary treatment. Additionally, processes like screening and grit removal usually take place in advance to remove materials that can damage or clog sewage pumps and lines of primary treatment clarifiers. Primary treatment involves temporarily holding the sewage in a quiescent basin, producing primary sediment and primary effluent for the subsequent process [1]. Secondary treatment is a biological treatment process for wastewater to achieve up to 90% organic content removal [1]. Aerobic and anaerobic processes are commonly used for secondary treatment. Different modes are applied in aerobic and anaerobic tank. Biological fluidized bed, trickling filter and rotating biocontactor are usually used in aerobic processes, while anaerobic upflow filter, anaerobic downflow filter and fluidized reactor are usually applied in anaerobic treatment [2]. In addition to the conventional activated sludge process, membrane bioreactor (MBR) is a recently developed wastewater treatment technique, which combines biological treatment with membrane filtration in a compact, single-step advanced process for wastewater treatment [3]. Compared to conventional activated sludge processes, MBRs have longer sludge retention times (SRT) since the membrane retains all solids in the reactor. Longer SRT increases the reactor biomass concentration, and utilisation by cells of stored materials and the extracellular polymeric substances (EPS) contribute to the pollutants removal [4].

Tertiary treatment is the final cleaning process that improves wastewater quality before it is reused, recycled or discharged to the environment. Tertiary treatment involves several different strategies for specific purposes, including filtration, tertiary lagoon and disinfection. Filtration can be achieved by media or membranes. Different membrane modules can be applied for tertiary treatment for different purposes, for example, reverse osmosis and nano filtration can be used for the removal of solutes and ultra-filtration and micro-filtration are used for the removal of fine particulates [5]. Disinfection usually can be implemented by chlorination, ozonation, or UV

radiation, which are capable to inactivate pathogenic microbes including bacteria, viruses, helminths and protozoans [1].

1.1.2 Wastewater sludge and its treatment

During the wastewater treatment processes, solid sediment produced from primary treatment and biomass generated during the secondary treatment are the major source for wastewater sludge generation. In a wastewater treatment plant, primary sludge is produced by settleable solids removed from raw wastewater in primary sedimentation, while secondary sludge, which is also called biological sludge, is generated by the biological processes such as activated sludge or biofilm system [6]. Wasted sludge must be treated and stabilized before disposal.

Aerobic sludge stabilisation is one of the sludge treatment processes, which is usually used in small wastewater treatment plants with a digestion time less than 25 days [6]. Microorganisms can aerobically degrade the organic matters to carbon dioxide, water and ammonia, and ammonia can be removed by nitrification subsequently. Normally, volatile suspended solid reduction during aerobic digestion can reach 30% at ambient temperature, and the reduction will increase under thermophilic conditions (40 – 55 °C) [7].

By the contrast, anaerobic digestion is usually applied for higher organic loading sludge treatment, particularly primary sludge. Anaerobic digestion is a series of biological processes in which microorganisms break down biodegradable material in the absence of oxygen, and it has been widely used in wastewater treatment plants for sludge reduction and stabilization. Anaerobic digestion can achieve effective destruction of pathogenic and faecal microorganisms, sufficient sludge reduction and production of sustainable resource (biogas) [8]. Four essential phases, namely hydrolysis, fermentation, acetogenesis and methanogenesis, are taken place to convert organic matters to biogas, which usually contains 60% of methane and 40% of CO_2. Compared to aerobic digestion, anaerobic digestion can achieve volatile solid removal up to 70%. Anaerobic digestion can be taken place under mesophilic conditions (33 °C - 35 °C) or thermophilic conditions (53 °C - 55 °C), and the optimal sludge retention times are 20-30 days and 12-15 days, respectively [9].

Composting is another bio-thermal aerobic process that decomposes the organic portion of the sludge, generating a large amount of heat [10]. It can reduce the wastewater sludge (by 25% approximately), reduce moisture of the sludge, as well as render the sludge harmless by converting

it into soil amendment material or fertilizer [10]. Furthermore, composting are also used for treatment of other waste, for example, winery waste [11], dairy processing sludge [12] and petroleum sludge [13].

1.1.3 Anaerobic digestion and processes improving digester performance

Anaerobic digestion is a complex biochemical conversion of organic matters to methane which involving a large amount of microbes. Different microorganism groups play significant role during hydrolysis, fermentation, acetogenesis and methaneogenesis, and the stability and efficiency of anaerobic digestion relies on the syntrophic relationship among microbial population [14-19]. The macroscopic condition variations would affect the digester performance via the effect on the microbial community structure. Mesophilic (33 – 35 °C) and thermophilic (53 – 55 °C) anaerobic digesters are showing different microbial community in compositions and biodiversity. For example, resesarchers found that *Bacterioidetes* and *Firmicutes* are dominating phyla in both mesophilic and thermophilic lab-scale digesters, while *Firmicutes* presented 70% and 40% of the composition in thermophilic and mesophilic digesters, respectively [20], and the order *Clostridiales* was the dominated order in mesophilic digester, while *Clostria* dominated in the thermophilic digester [20]. Another study also revealed that the optimum temperature range for methanogenesis is 30-35 °C, and low temperature (below 15 °C) would inhibit methanogenesis process [21]. SRT was also reported to affected the microbial population that *Chloroflexi* and *Syntrophomonas* were decreasing, while *Bacteroidetes* was increasing when SRT decrease from 20 to 4 days [22].

There are several parameters used for indicating the performance of anaerobic digestion. Sludge parameters, such as total solid content (TS), volatile solid content (VS), total chemical oxygen demand (tCOD) and soluble chemical oxygen demand (sCOD) are major parameters to indicate organic matters and their removals. Parameters including digestate pH, alkalinity and volatile acids concentration can demonstrate volatile fatty acid accumulation during anaerobic digestion. As the major product of anaerobic digestion, biogas production and composition also are important indicators for digestion performance. The Table 1.1 listed the ranges of several parameters in a typical anaerobic digestion.

Table 1.1 Sludge parameter ranges for the typical anaerobic digester.

Parameter		Range for the typical anaerobic digester	Reference
tCOD removal		40 – 60%	[23-25]
sCOD removal		70 – 90%	[26, 27]
Digestate pH		6.6 - 7.4	[28, 29]
Alkalinity (at pH=4.3)		2000 – 4000 mg $CaCO_3$/L	[28, 30]
ratio of total volatile fatty acid/alkalinity		0.1- 0.35	[30]
Biogas production		0.75-1.12 m^3/kg $VS_{reduction}$	[28]
Biogas composition	Methane	50 – 75%	[28]
	CO_2	25 – 50%	
	H_2	0 – 1 %	
	H_2S	0 – 3%	

In order to improve the anaerobic digestion performance, studies have introduced several processes for enhanced digestion performance. Pre-treatment, which can improve the degradability of the complex material therefore the hydrolysis rate of the anaerobic digestion, has been widely studied. Several pretreament methods including biological, thermal hydrolysis, mechanical treatment, ozonation and alkali treatment were reported in literatures. Thermal pre-treatment (150 – 180 °C for 30 – 60 mins) can partially transfer particulate organic matters into soluble phase, which enhances the anaerobic digestion [31]. Studies showed that thermal pretreament under 170 °C for 30 mins could improve the biogas/methane production by 30 – 50% in pilot plants [32, 33], and up to 80% in batch test [34].

Apart from the pre-treatment, another modified anaerobic digestion process, namely recuperative thickening, was also reported to be able to enhance the digestion performance. Recuperative thickening was first demonstrated by Torpey and Melbinger in 1967 [35], during which digestate was partially thickened and returned to the digester. As a result, recuperative thickening allows extention of SRT from hydraulic retention time (HRT) and returns active bacterial to the digester. It has been approved in full-scale plants [36, 37] as well as lab-scale digesters [38, 39] that

recuperative thickening could improve the biogas production, volatile solid (VS) removals and sludge dewaterability. Recuperative thickening is also of great interest for recent researchers, because it is able to increase the digester capacity without extension of digestion and higher physical footprint, which will fulfil the demand of larger treatment capacity plants due to urbanization and population growth.

However, by introducing additional thickening processes to the digester, oxygen exposure and shearing may influence the anaerobic digester's performance. Effect of oxygen exposure during thickening process on the digester performance was studied by a few researchers [37, 40], and the results showed that low oxygen exposure during the thickening process had no appreciable effect on the methanogens inactivity [37] and minimal oxygen during gravity belt thickening did not affect the biogas production of the anaerobic digestion [40]. On the other hand, thickening process like centrifuge and rotatory drum, would ineluctably lead to cell lysis coursed by shearing. Some studied observed significant increase in methane production when centrifuge thickening was applied [41-43]. However, other studies observed negligible or negative impact of centrifuge thickening process on the methanogenic sludge viability and activity. Batstone et al. [44, 45] found that the high-speed centrifuged used in full-scale digesters reduced the specific methanogenic activity of digestate particularly at high solid. Similar results were observed by Deveci [46] that shear forces would cause the loss in the viability of bacterial population when solid content was above 10%. The microbial community stability and robustness affected by shearing could be the main reason for the digestion performance reduction. Some studies found that the density of granules after sheared digester remained unchanged [47], while other studies reported archaea and bacteria were observed with significant reduction in the abundance and diversity under high hydrodynamic shear [48, 49].

1.1.4 Pollutants management for wastewater sludge

Due to the high nutrients content of the treated sewage sludge (biosolids), biosolids are usually sent to land application for agriculture land. Australia currently produces approximately 300,000 dry tonnes of biosolids annually. Approximately 55% is applied to agricultural land and around 30% is disposed of in landfill or stockpiled [50]. However, pollutants residue in the biosolid must be considered before land application from the perspectives of community health and environmental justice [51, 52].

Foul odour has been frequently reported to be the major concern when the biosolids are applied to farm land, and the odour components are usually complained by the residents for causing physical symptoms such as respiratory distress, headaches, and skin rashes [52, 53]. Odours from wastewater sludge arise as a result of bacterial activity, and major odorous compounds include hydrogen sulphide, ammonia, amines, mercaptans, organic acid and skatoles [54, 55]. Currently, there are five odour treatment methods available for sludge odour control for WWTPs, which are wet scrubbing, activated carbon absorption, activated sludge scrubbing, bio-scrubbing and biofiltration [56].

Trace organic contaminants (TrOCs) are emerging pollutants in wastewater and wastewater sludge, which may have negative effects on the environment and/or organisms. TrOCs include several groups of compounds consumed in household and industry, like pharmaceutical compounds, personal care products, hormone, phytosanitary products, insecticides, etc. TrOCs can transfer to the sewage sludge during the wastewater treatment processes (primary and secondary clarification) [57-59]. As a result, TrOCs in municipal wastewater sludge can be detected in both aqueous (several μg/L or more) and solid phase (several μg/kg dry weight or more). Antibiotics and pharmaceutically active compounds are amongst the most investigated TrOCs in digested sludge. Although TrOCs are detected in very low concentrations in municipal sewage, some of these TrOCs have the potential to cause chronic disorders in animals and humans at a sufficient concentration. Several countries have already imposed controls on certain TrOCs such as nonylphenol (NP) and nonylphenol ethoxylates (NPEs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and dibenzo-p-furans (PCDD/Fs) [60]. Therefore, it is necessary to understand the fate of TrOCs during sludge treatment process pollutants control. Several compounds were found to be well removed from lab-scale anaerobic digesters, such as trimethoprim [61, 62], citalopram [62], sulfamethoxazole [61, 63], caffeine [61], naproxen [64], diclofenac [64], estrone [64, 65], 17α-ethinylestradiol [64, 65]. However, other compounds like fluoxetine [61, 63], carbamazepine [61, 62] and iopromide [64] were resistant to anaerobic digestion. It is important to note that most previous studies involved the spiking (artificial addition) of TrOCs to the feed sludge at elevated concentrations. Only limited studies force on the environmental concentrations of TrOCs wastewater sludge and their removals during the anaerobic digestion.

1.2 Statement of the problem

Recuperative thickening has been approved in full-scale wastewater treatment plants to enhance the organic conversion to methane and volatile solid reduction [66-68]. However, previous data were obtained mostly from full-scale operation, where there could be many factors in play that could also influence anaerobic digestion performance. Few of these previous studies have attempted to understand the underlying mechanisms of recuperative thickening to enhance anaerobic digestion performance and present the optimised recuperative thickening process. Additionally, pre-treatment, as a feasible procedure for full-scale plants, were also limited studied in lab-scale continuous anaerobic digesters. Therefore, this project aims to experimentally assess the effect of recuperative thickening and thermal pretreament on the anaerobic digestion. Particularly, this project will evaluate the use of recuperative thickening to increase treatment capacity and efficiency with respect to a range of performance parameters including biogas production, VS and COD reduction, process stability, and biosolids odour. Furthermore, TrOCs, as emerging pollutants to water environment, were also limitedly studied for their occurrence and removals during wastewater sludge treatment processes. It will be of great importance to reveal the TrOC occurrence in the wastewater sludge and study their fate during the anaerobic digestion.

1.3 Objectives of the research

This project aims to assess the modified anaerobic digestion process on the digestion performance improvement, as well as the TrOCs fate during such processes.

The key objectives of this project are to

1. Assess the effect of SRTs on the anaerobic digestion performance for conventional anaerobic process when SRT and HRT are concordant.
2. Examine the anaerobic digester performance improvement by recuperative thickening on the digestion performance when HRT remained unchanged.
3. Evaluate the influence of sheared thickening process on the digestion performance and microbial community shift when recuperative thickening applied.
4. Compare two of the enhancement approaches of anaerobic digestion, thermal pretreament and recuperative thickening, from the perspective of digester performance.

5. Reveal the TrOCs occurrence from wastewater sludge and elucidate their fates during anaerobic digestion at different conditions.

1.4 Thesis outline

The following Figure 1.1 outline the structure of the thesis.

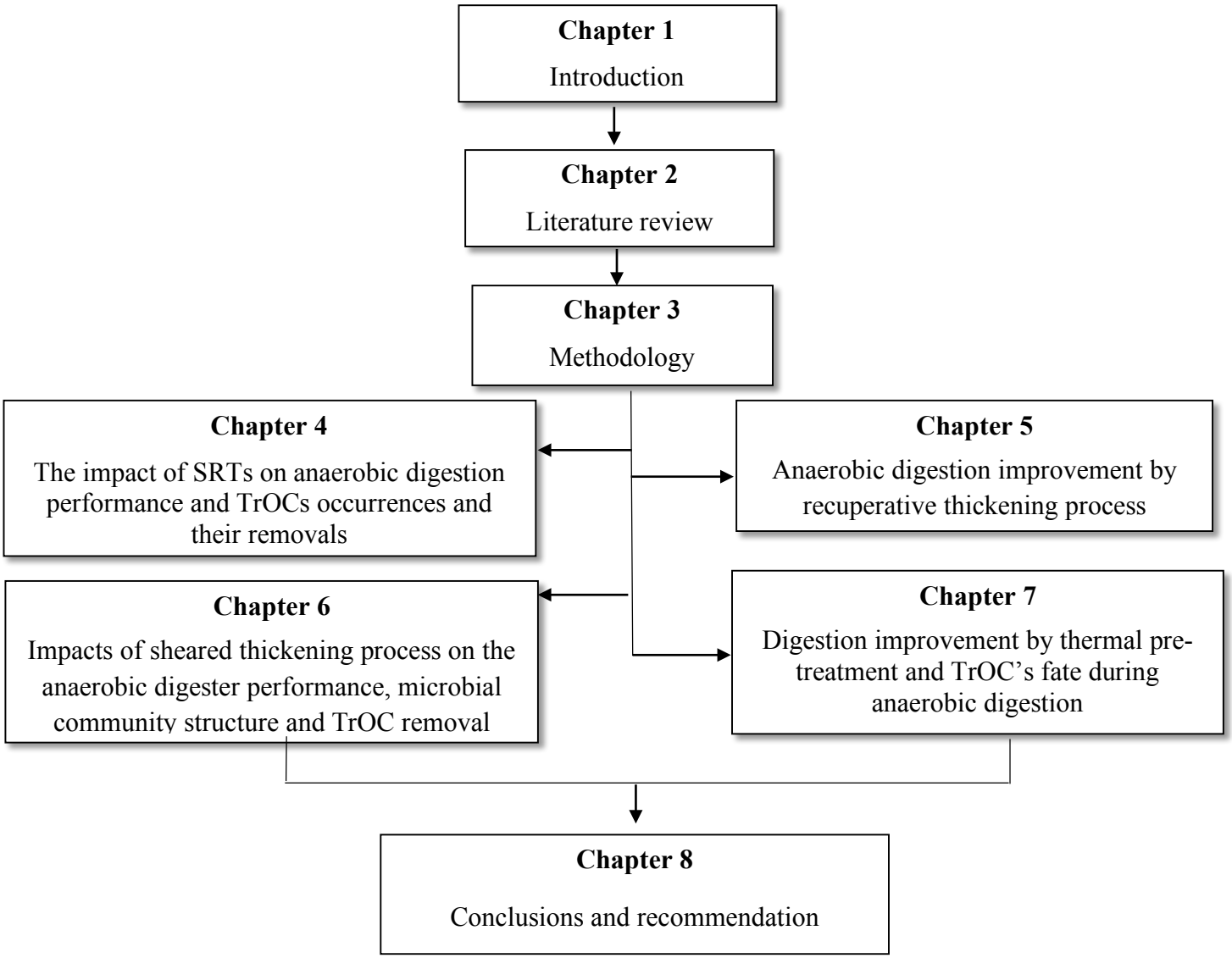


Figure 1.1 Thesis outline.

Chapter 2 Literature review

Wastewater sludge is a term referring to the residual, semi-solid material that is produced during sewage treatment of industrial or municipal wastewater. Sludge generated in wastewater treatment plants (WWTPs) normally amounts to a small portion (around 1%) of the inflow [6]. However, given the very large inflow of up to 200 L/(equivalent person.day), the quantity of sludge is very significant. The amount of sludge produced in the modern society is increasing due to population growth, urbanization and upgrading of WWTPs as mandated by environmental legislation. According to historical data, the amount of sludge generated in the EU was 10 million tons in 2005, and USA produced 6.4 million tons of sludge in 1998 [69, 70]. In Asia, China generated 11.2 million tonnes of dry sludge in 2010 [71]. In Australia, dry sludge production from wastewater treatment increased by about 3% each year from 0.30 million tonnes in 2010 to 0.33 million tonnes in 2013 [71]. More importantly, the sludge generation is not evenly distributed geographically, and the urban areas, with limited land area, are facing critical problems from increasing sludge disposal. Therefore, the need to reduce the amount of sludge produced in WWTPs requires variety of solutions and techniques to be implemented on-site. This chapter reviews the current sludge treatment techniques especially anaerobic digestion, a few modified processes for digestion performance improvement, and pollutants management during sludge treatment.

2.1 Wastewater treatment

Wastewater treatment, also called sewage treatment, generally involves three stages, which are primary, secondary and tertiary treatment. Necessary pre-treatment is also required in advance to remove materials that can damage or clog sewage pumps and lines of primary treatment clarifiers. Pre-treatment includes simple processes such as screening, grit removal to remove most of the solids, which is also called primary sedimentation [1]. Primary sedimentation is based on the concept of gravitational separation, which is affected by several factors, such as surface overflow rate and total suspended solid concentration of the influent [72]. As reported, higher overflow rate will result in lower suspended solid removal, and higher suspended solid concentration will lead to higher solid removal efficiency [72]. The sedimentation from primary treatment is call primary sludge, which is objectionable and has a solids content of 1-2%.

Secondary treatment is to degrade up to 90% organic content of wastewater by biological treatment [1]. Attached growth process and suspended growth are two most common methods applied in secondary treatment [1]. Attached-growth processes are biological processes used for water neutralization, in which the microorganisms attach to some inert solid surface to form a biofilm, which is responsible for the conversion of organic matters or other constituents [73]. Different modes are applied in aerobic and anaerobic tank. Biological fluidized bed, trickling filter and rotating biocontactor are usually used in aerobic processes, while anaerobic upflow filter, anaerobic downflow filter and expanded/fluidized reactor are applied in anaerobic treatment [2]. Suspended grow process is normally dominated by aerobic bacteria and protozoa, which form the biomass called activated sludge [1]. Activated sludge is a biological contact process where bacteria, fungi, protozoa and small organisms such as rotifers and nematode worms are capable to aerobically stabilize the organic content of wastewater. Among them, bacteria are the most important group of microorganisms, since they can form the structural and functional activity of the activated sludge floc. The predominate type of bacteria present will be determined by the nature of the organic substances in the wastewater, operation condition of the plant, and the environmental conditions in the process [74]. Activated sludge is able to oxidize carbonaceous biological matter, convert ammonia to nitrite or nitrate, remove phosphates and metals [75]. In addition to the conventional activated sludge process, membrane bioreactor (MBR) is a recently developed wastewater treatment technique, which combines biological treatment with membrane filtration in a compact, single-step advanced process for wastewater treatment [3]. The membranes or modules applied in MBRs include reverse osmosis (RO) and nanofiltration (NF) for the removal of solutes and ultrafiltration (UF) and microfiltration (MF) for the removal of fine particulates [5]. Compared to conventional activated sludge processes, MBRs have longer SRT since the membrane retains all solids in the reactor.

Tertiary treatment involves several different strategies for specific purpose, including filtration, tertiary lagoon and disinfection. Filtration can be achieved by media or membranes. Sand, as a filtration medium, has been reported to efficiently remove phosphorus [76], some pharmaceutical compounds [77, 78], coliform bacteria and coliphage [79]. Additionally, different membrane modules can be applied for tertiary treatment for different purposes, for example, RO and NF for the removal of solutes and UF/MF for the removal of fine particulates [5]. Activated carbon is also used as a media for tertiary filtration, which has been revealed to remove resistant organic materials,

such as dyes [80]. Tertiary lagoon has been reported to be effective on nitrogen removal by volatilisation of ammonia and sedimentation of organic nitrogen, while little phosphorus removal was observed [81]. Disinfection can be achieved by chlorination, ozonation, or UV radiation, which are capable to inactivate pathogenic microbes including bacteria, viruses, helminths and protozoans [1].

2.2 Treatment of wastewater sludge

2.2.1 Sludge characteristics

In a wastewater treatment plant, various kinds of sludge are produced by different treatment units. Primary sludge is produced by settleable solids removed from raw wastewater in primary sedimentation; while secondary sludge, which is also called activated sludge, is generated by the biological processes such as biological nutrient remove reactor or biofilm system [6]. The production of primary sludge is related to the amount of settleable solids in raw wastewater, 50-65% of which can be assumed to be settled to form primary sludge [82]. Activated sludge is formed by heterotrophic biomass growth, which converts organic biodegradable matter to new cellular biomass, and the maximum growth yield can reach 0.6-0.7 in aerobic condition [6]. A simplified scheme (Figure 2.1) indicates the processes leading to biological sludge production.

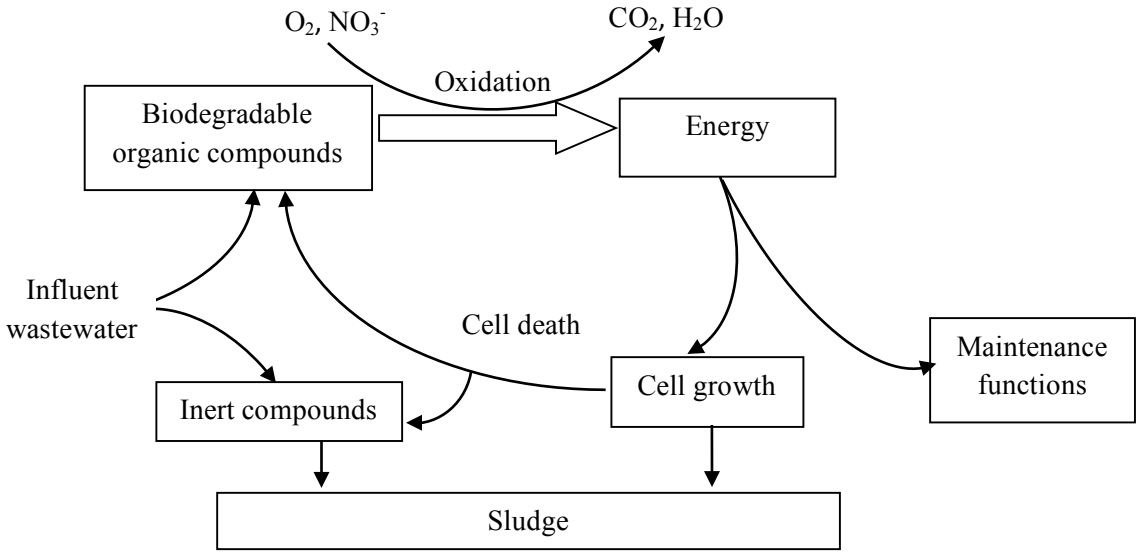


Figure 2.1 Simplified schemes of the processes leading to biological sludge production.

Several parameters can indicate the characteristics of sludge, including total solid (TS), volatile solids (VS), total chemical oxygen demand (tCOD), soluble chemical oxygen demand (sCOD)

alkalinity etc. However, the composition of the sludge may vary in terms of different types. The typical chemical composition and property of different sludge is reported in Table 2.1 [70].

Table 2.1 Typical chemical composition and properties of wastewater sludge [70].

Property	Primary sludge		Digested sludge		Activated sludge
	Range	Typical	Range	Typical	Range
Total solid (TS), %	2.5-5.5	3.5	2.0-8.0	5.0	0.83-1.16
Volatile solid (% of TS)	60-80	65	30-60	40	59-88
Grease and fats (% of TS)					
Ether soluble	6-30	-	5-20	18	-
Ether extract	7-35	-	-	-	5-12
Protein (% of TS)	20-30	25	15-20	18	32-41
Nitrogen (N, % of TS)	1.5-4	2.5	1.6-6.0	3.0	2.4-5.0
Phosphorous (P_2O_5, % of TS)	0.8-2.8	1.6	1.5-4.0	2.5	2.8-11.0
Cellulose (% of TS)	8.0-15.0	10.0	8.0-15.0	10.0	-
Iron (not as sulfide)	2.0-4.0	2.5	3.0-8.0	4.0	-
Silica (SiO_2, % of TS)	15.0-20.0	-	10.0-20.0	-	-
Alkalinity (mg/L as $CaCO_3$)	500-1500	600	2500-3500	-	580-1100
Organic acids (mg/L as acetic acid)	200-2000	500	100-600	300	1100-1700
pH	5.0-8.0	6.0	6.5-7.5	7.0	6.5-8.0

Table 2.1 compares the parameters of primary and anaerobically digested sludge. Digested sludge has higher alkalinity and pH, and lower volatile solid ratio than primary sludge and activated sludge. According to Gore [83], degradation of organic acid and nitrogen based compounds in acid regression is responsible for the pH increasing, and degradation of proteins and amino acid in methane fermentation is associated with the increase of alkalinity. The slowly growing acetogenic bacteria can oxidize volatile acid into acetate acid, molecular hydrogen, and carbon dioxide that are suitable as substrates for the methanogenic bacteria in anaerobic digestion [84], which leads to the reduction of volatile solids in the digested sludge.

Vesilind [85] observed that the physical characteristics of sludge, such as particle size distributions and dewatering characteristic, are also varied between different sludge, even with roughly equal total solid concentrations (Table 2.2). The dewatering characteristic is related to the sludge particle size distribution, and the colloidal solids have the greatest effect on dewatering [85]. Vesilind [85] explained that, the digested sludge with a high specific resistance to filtration is difficult to dewater, while a low capillary suction time suggested that the raw primary sludge is readily dewatered (Table 2.2).

Table 2.2 Characteristics of primary sludge and anaerobically digested sludge [85].

	Primary sludge	Digested sludge
Specific resistance (m/kg)	2.1×10^{14}	9.3×10^{14}
Capillary suction time (s)	17	144
Total solids (mg/L)	9698	10266
Rigid settleable solids (% of TS)	66.5	32.9
Fragile settleable solids (% of TS)	23.9	39.5
Supracolloidal solids (% of TS)	3.7	19.5
True colloidal solids (% of TS)	0.5	2.9
Dissolved solids (% of TS)	5.4	5.2

2.2.2 Wastewater sludge treatment

Biological processes, which are based on heterotrophic bacteria and autotrophic bacteria, are usually applied for wastewater sludge treatment. Table 2.3 summarises two groups of bacteria that are involved with biological processes in WWTPs. The heterotrophic bacteria are the predominant group of organisms, which are fed mainly on organic carbon molecules rather than inorganic ones. By contrast, the autotrophic bacteria take in inorganic chemicals, and use these in the synthesis of organic compounds. Nitrifying bacteria are the most important autotrophic bacteria, which can convert ammonia to nitrite or nitrate in wastewater. Autotrophs are usually out-competed by heterotrophs due to low growth rates [86].

Table 2.3 Synthetic classification of microorganisms involved in biological processes [6].

Group	Origin of cell carbon	Energy source/ electron donor	Electron acceptor	
Heterotrophic bacteria	Organic compounds	Organic compounds	Aerobic Anoxic Anaerobic	Oxygen Nitrate, sulphate Organic compounds
Autotrophic bacteria	Inorganic compounds	Ammonia, H_2S, Fe^{2+}	Oxygen	

Aerobic sludge stabilisation is the most common process for sludge treatment in small WWTPs with a digestion time less than 25 days [6]. Microorganisms can aerobically degrade the organic matters to carbon dioxide, water and ammonia, and ammonia can be removed by nitrification subsequently. Normally, volatile suspended solid reduction during aerobic digestion can reach 30% at ambient temperature, and the reduction will increase under thermophilic conditions (50 – 55 °C) [7].

On the other hand, anaerobic digestion is most widely used in larger WWTPs; since the product (biogas) can provide economical energy for the sites. Anaerobic digestion has other benefits, such as effective destruction of pathogenic and faecal micro-organisms, sufficient sludge reduction, and increment of proportion of nutrients in sludge which can be used as fertilizer [8]. Anaerobic digestion is achieved through four major phases: hydrolysis, fermentation, acidogenesis and methanogenesis, which is usually performed under mesophilic conditions (33 -35 °C) [6]. The rate limiting step is the hydrolysis of solid, and it is the reason that many studies have focused on the pre-treatment methods to increase the hydrolysis rate and sludge degradation. Compared to aerobic digestion, mesophilic anaerobic digestion can reduce more VS (40% recudtion) [6]. Some studies also focused on thermophilic anaerobic digestion (53 - 55 °C), which found that lower solid retention time (SRT) (12 - 15 days) and higher biogas production rate could be achieved under thermophilic conditions [9].

Composting is a bio-thermal aerobic process that decomposes the organic portion of the sludge, generating a large amount of heat [10]. Usually, wastewater sludge will be mixed with other carbon sources like sawdust, straw or wood chips, and bacteria can consume both the wastewater solid and the added carbon sources. Turovskiy and Westbrook [10] reported that composting can reduce

the wastewater sludge (by 25% approximately), reduce moisture of the sludge, as well as render the sludge harmless by converting it into soil amendment material or fertilizer. Furthermore, composting are also used for treatment of other waste, for example, winery waste [11], dairy processing sludge [12] and petroleum sludge [13].

Table 2.4 Overview of sludge treatment methods

Treatment	Substrate	Typical SRT	Solid reduction	Products
Aerobic digester	Wastewater sludge	<25 d	VSS reduction 30% at 25 °C;	CO_2, water and ammonia (followed by nitrification)
Anaeorbic digestion	Wastewater sludge	25-30 d at 33-35 °C; 12-15 d at 53-55 °C	VS reduction 40% at 33-35 °C	CO_2, CH_4, and trace of H_2S
Composting	Sludge and other carbon sources (sawdust, straw)	N.A.	25% of solid	CO_2, water, organic matter (carbon, protein, humus, and chemical energy)

2.3 Anaerobic digestion

2.3.1 Biochemical interactions and microbial community of anaerobic digestion

The anaerobic biochemical conversion of organic matters to methane is a complex biogenic process involving a large amount of microbes. As shown in Figure 2.2, direct and indirect symbiotic associations are involved in the overall conversion of organic carbon to biogas, which can be recognized as nine steps [87]:

(1) Enzymatic hydrolysis of organic polymers to intermediate organic monomers such as sugar, fatty acids, and amino acids;

(2) Fermentation of organic monomer to hydrogen, bicarbonate, pyruvate, alcohols and lower fatty acids;

(3) Oxidation of reduced organic products to bicarbonate and acetate by obligate hydrogen-producing acetogens;

(4) Acetogenic respiration of bicarbonate by homoacetogens;

(5) Oxidation of reduced organic products to bicarbonate and acetate by nitrate-reducing bacteria and sulphate-reducing bacteria;

(6) Oxidation of acetate to bicarbonate by nitrate-reducing bacteria and sulphate-reducing bacteria;

(7) Oxidation of hydrogen by nitrate-reducing bacteria and sulphate-reducing bacteria;

(8) Aceticlastic methane fermentation;

(9) Methanogenic respiration of bicarbonate

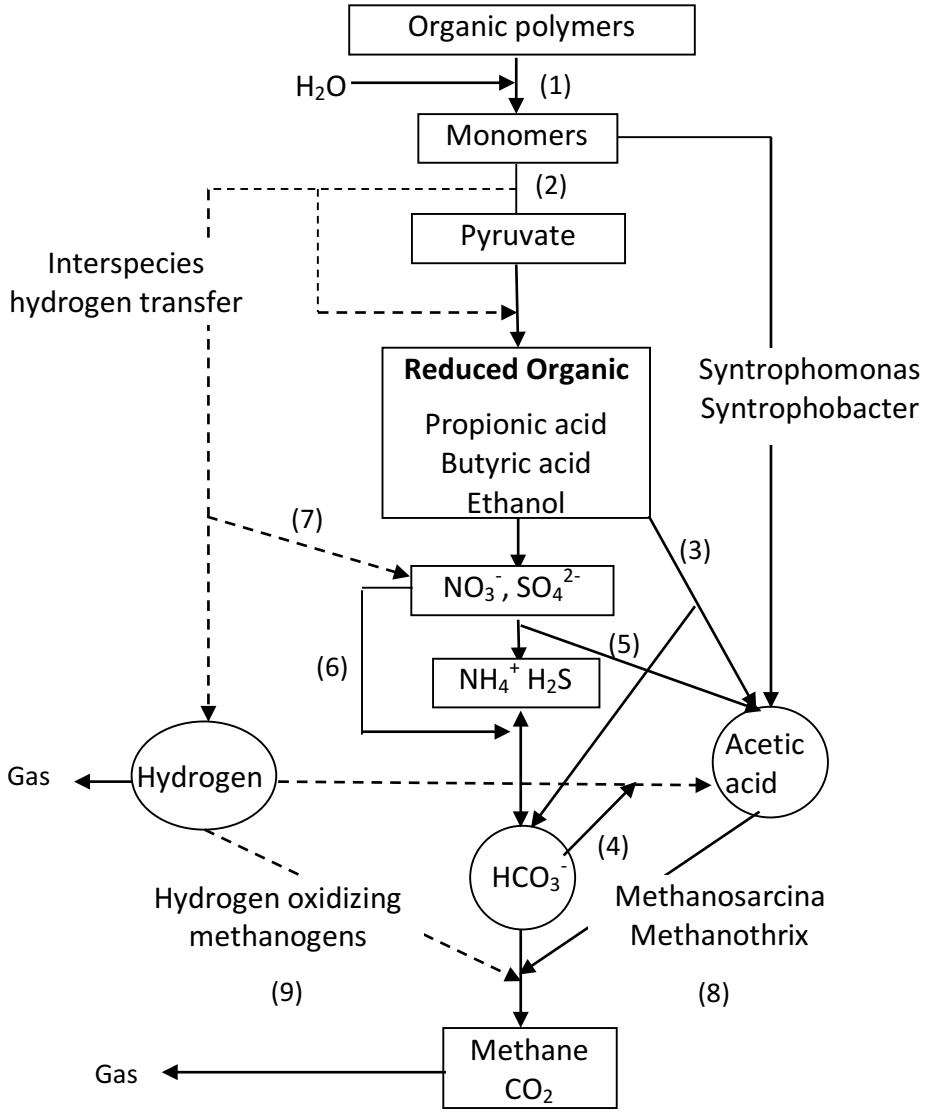


Figure 2.2 Substrates conversion during anaerobic treatment.

In the hydrolysis step, complex organic compounds and colloidal matters are converted into their monomer or dimeric components, such as amino acids, single sugars and long chain fatty acids. Two main mechanisms are considered to be responsible for release of enzymes and hydrolysis of the complex substrate [88]:

1) The microorganism secretes enzymes to the bulk liquid, where they will either adsorb to a particle or react with the soluble substrate;

2) The microorganism attaches to the particle, secretes enzymes into the vicinity of the particle and then the microorganism will benefit from the released dissolved substrates, for example, amino acid, sugar, free long chain fatty acid and glycerol.

Different substrates, for example, proteins, carbohydrates and lipids, have distinguished enzymatic hydrolysis pathways, as indicated in Figure 2.3.

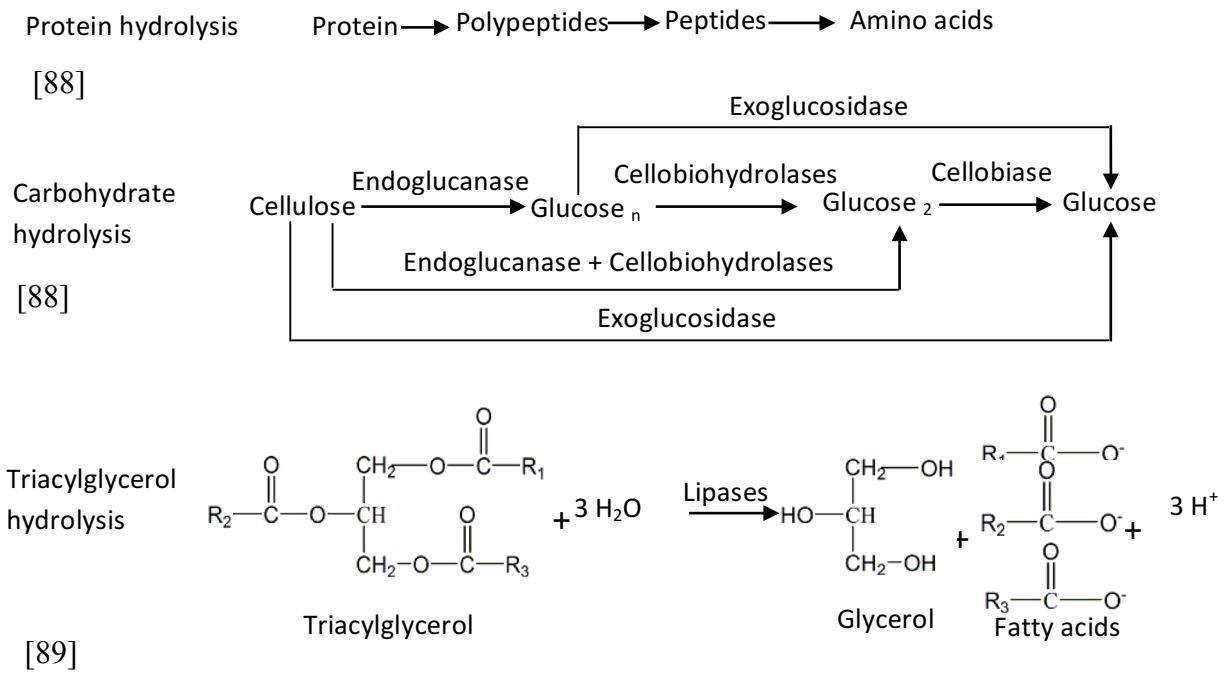


Figure 2.3 Enzymatic hydrolysis pathways of proteins, carbohydrates and lipids.

Microorganisms secrete proteinases when concentration of amino acid and inorganic nutrients in the water are low or protein and peptides concentrations are high [88]. The inhibitors of proteinase production include amino acids, high inorganic phosphate levels and glucose [90]. In addition, the hydrolysis of carbohydrates (cellulose) is driven by a mixture of cellulolytic enzymes, for example, exo-glucanases, endo-glucanases and cellobiases (Figure 2.3). Similar to proteinases, the production of cellulolytic enzymes is inhibited by high glucose levels, and stimulated by low glucose levels [88]. However, amino acid was reported to have no effect on the production of cellulolytic enzymes [90]. The lipids in wastewater are usually presented as triacyglycerides, whose hydrolysis is executed by triglyceride lipases (Figure 2.3). Hydrolysis of triacyglycerides usually leads to form soluble long chain fatty acid, however, the long chain fatty acid may cause

severe inhibition of methanogenic and acetogenic bacteria growth and reduce degradation of the long chain fatty acid [89].

Followed by hydrolysis, the hydrolysis products are converted into acetic acid and other volatile fatty acids and alcohol during acidogenesis. Then fatty acid and alcohol will convert to acetic acid or hydrogen and carbon dioxide during the acetogenesis. Acetic acid, carbon dioxide plus hydrogen and methanol are the main substrates for the methanogenesis to form methane and carbon dioxide [88].

As described before, the anaerobic digestion is a microbial mediated process, therefore, the stability and efficiency of anaerobic digestion rely on the syntrophic relationship among microbial population including hydrolysing and fermenting bacteria, specialized acidogenic and acetogenic syntrophs, and methanogenic archaea [14-19]. Anaerobic cellulolytic bacteria hydrolyse cellulose to soluble sugars, which can be utilized by acidogenic bacteria. Acetogenic and/or acidogenic bacteria produce acetate and/or (H_2 + CO_2), which can be coverted to methane by methanogen [18]. Studies have revealed that cellulose hydrolysers include the order *Halanaerobium* [17], the order *Clostridiales* and *Bacteroidales* [15] and the genus *Acetivibrio* [18]. The *Clostridia* class and the *Bacteroidaceae* family [17] performed in the acidogenic process; and genus *Clostridium*, *Treponema*, *Eubacterium*, *Thermoanaerobacter, Moorella* [17], *Methanosaeta* [14] and *Porphyromonadaceas* [18] are the dominant acetogenic bacteria. On the other hand, microbial community structure was found unique and resilient in full-scale anaerobic digesters [16]. By analysing over 100 samples from 9 full-scale systems, Werner et al. [16] concluded that ecological dynamics of syntrophic populations were stable, resilient, and highly selective along environmental gradients, and communities with greater evenness had a higher methanogenic activity. Furthermore, environmental factors (e.g. operating conditions and process configurations) could lead to the variability in structure and function of microbial population, hence the performance of anaerobic digester system. The microbial diversity such as community evenness was demonstrated as indicator for stability and robustness of the community function [15, 16].

The microbial community structure can be affected by several factors that imposed to anaerobic digestion. For example, microbial community population is showing different compositions and biodiversity in mesophilic and thermophilic anaerobic digesters. Moset et al. [20] found that *Bacterioidetes* and *Firmicutes* presented the dominating phyla in both mesophilic and thermophilic

lab-scale digesters, while *Firmicutes* presented 70% and 40% of the composition in thermophilic and mesophilic digester, respectively. In terms of the phylum *Firmicutes* community composition, *Clostridiales* was the dominated order in the mesophilic digester, while *Clostria* dominated in the thermophilic digester [20]. A study conducted by Gagliano et al. [91] reported that the Shannon Weaver diversity and Pielou's evenness indexes both decreased under thermophilic conditions, indicating that thermophilic anaerobic biomass could be more susceptible to sudden changes and less able to adapt to operative variations. In addition, Donoso-Bravo et al. [21] studied the effect of temperature on methanogenesis, which found that the optimum temperature range for methanogenesis is 30-35 °C, and the low temperature (below 15 °C) would inhibit methanogenesis process. SRT was also reported to affected the microbial population [22] while HRT had little impact [20]. Lee et al. [22] found a significant bacterial population shift associated with SRT decreasing from 20 to 4 days: *Chloroflexi* and *Syntrophomonas* were decreasing, while *Bacteroidetes* and two acetogenic genera belonging to the phyla *Firmicutes* and *Spirochaetales* were increasing. Furthermore, other operation interference like shearing could influence the microbial community structure as well. Shearing is inevitable when thickening process or intensive mixing is taken place during the anaerobic digestion. In a study by Kundu et al. [48], hydrodynamic shear (upflow velocities from 4 m/h up to 10 m/h) was applied to a mesophilic hybrid anaerobic reactor. Archaea and bacteria were observed with significant reduction in the abundance and diversity under high upflow velocity (>6 m/h). Among all methanogenic groups, *Methanosaetaceae* was mostly affected due to breakage and wash out of granules [48]. Microbial community and granules were also affected by the shear in the continuously stirred anaerobic digester [47, 49]. Hoffmann et al. [49] found that different mixing intensities ranging from 250 to 1500 rpm influenced the competition between the acetoclastic methanogens, *M. concilii* and *Methanosarcina spp*.. *Methanosarcina spp*. became more important in the intensely mixed digesters that could result in more stable digesters. Therefore, increased mixing intensities could positively affected the long-term stability [49]. Jiang et al. [47] applied a continuous hydrodynamic shear to a continuous stirred tank reactor (CSTR). The shape of original sludge granules was observed to change from approximately ellipsoidal to elongated and flattened. It is also found that the density of granules after sheared digester remained unchanged, and the mechanical resistance of deformed sludge granules was slightly enhanced [47].

2.3.2 Factors affecting anaerobic digestion

2.3.2.1 Pre-treatment

Enzymatic hydrolysis is the first and rate limiting step of anaerobic digestion, therefore, many studies have focused on the pre-treatment prior to the anaerobic digestion in order to improve the inherent degradability of the complex material and increase digestion rate. Several pre-treatment methods have been reported, including biological, thermal hydrolysis, mechanical treatment, ozonation and alkali treatment.

Biological pre-treatment can enhance the hydrolysis process in an additional stage before the main digestion process. The most common method is temperature-phased anaerobic digestion, which uses a higher temperature stage at either thermophilic (55 °C) or hyper-thermophilic (60-70 °C) conditions [92]. Thermophilic conditions generally benefit the organic solid destruction rate, contributing to the higher hydrolytic activity.

Table 2.5 summarises the effect of thermal or hyper thermal biological pre-treatment on methane production in anaerobic digestion. Temperature biochemical pre-treatment allows significant increase in methane production during the anaerobic digestion. Skiadas et al. [93] compared different sludge under same pretreament and anaerobic digestion condition, and results showed that activated sludge was more impressionable to thermophilic pre-treatment, which helped to increase the methane production by 25-50%. Hyper-thermophilic pre-treatment is also the option [93-95], which can increase the methane production by 11-58%.

Table 2.5 Effect of thermal or hyper thermal biological pre-treatment on anaerobic digestion.

Substrate	Pre-treatment	Anaerobic digestion condition	Increased CH_4 production (%)	Reference
Primary sludge	50-65 °C, 2 days	Continuous reactor, 35 °C, HRT=14 days	25	[96]
Primary sludge	70 °C, 2 days	Continuous reactor, 55 °C, HRT=13 days	11	[93]
Primary sludge	70 °C, 2 days	Continuous reactor, 55 °C, HRT=13 days	48	[94]

Substrate	Pre-treatment	Anaerobic digestion condition	Increased CH_4 production (%)	Reference
Activated sludge	Microaerobic, 60-70 °C, 1 day	Batch test, 37 °C, 10 days	50	[97]
Activated sludge	Microaerobic, 65 °C, 1 day	Continuous reactor, 35 °C, HRT=21 days	0	[98]
Activated sludge	70 °C, 2 days	Continuous reactor, 55 °C, HRT=13 days	28	[93]
Activated sludge	70 °C, 9 hours	Batch test, 55 °C	58	[95]

Thermal hydrolysis is another treatment improves methane production, where sludge is boiled under high temperature and high pressure. Thermal hydrolysis can partially transfer particulate organic matters into soluble phase, which enhances the degradability of organic matters during anaerobic digestion [31]. Schieder et al. [99] demonstrated that increasing the pressure and temperature of thermal hydrolysis can lead to breakdown of organic part of the waste into short-chain fragments, which are better suited for biological digestion by microorganisms. As reported in previous studies [100-104], the optimal temperature of thermal hydrolysis is 160-180 °C and treatment duration is 30-60 mins. Table 2.6 reports the influence of thermal hydrolysis on the anaerobic digestion in the lab-scale experiments, pilot plants, as well as in WWTPs. In fact, thermal hydrolysis helps to increase biogas/methane production of anaerobic digestion, and it also results in increased hydrolysis rates [100, 103]. Perez-Elvira et al. [105] reported that, thermal hydrolysis led to higher biogas yield with decreased HRT, which indicated that thermal hydrolysis can increase the sludge digestibility. Additionally, thermal hydrolysis treatment has positive effects on sludge sanitation by reducing pathogen, and can reduce sludge viscosity for the subsequent sludge handling [106]. Thermal hydrolysis was also applied in WWTP [107], and the energy balance calculation of the practical experience showed that net electricity production increased by 20% due to more biogas production although the thermal hydrolysis process consumed more energy.

Table 2.6 Effect of thermal hydrolysis on the anaerobic digestion.

Substrate	Pre-treatment	Anaerobic digestion condition	Result	Ref.
Activated sludge	170 °C, 60 mins	Batch test, 35 °C, 23 days	Biogas production increased by 45%	[101]
Activated sludge	170 °C, 60 mins	Continuous reactor, 35 °C, HRT=20 days	Biogas production increased by 54%	[101]
Activated sludge mixed with cattle dung	121 °C, 30 min	Batch test, 37 °C, 7 days	Biogas production increased from 3657 L/m^3 to 4843 L/m^3 sludge	[108]
Municipal waste sludge	170-175 °C, 60 min	Pilot plant, 35-37 °C, HRT=35 days	Biogas production didn't increase after pre-treatment; however, higher digestion rate and lower volatile fatty acids were accumulated	[100]
Activated sludge	175 °C, 30 min	Pilot plant, HRT=17 days	Biogas yield increased by 33%, VS removal increased by 30%	[32]
Activated sludge	180 °C, 60 min	Pilot plant, HRT=20 days	Accumulated biogas production increased by 80% during 7 days	[109]
Mixed sludge	170 °C, 30 min	Pilot plant, HRT=12 days	Methane production increased by 55%	[33]
Mixed sludge	170 °C, 30 min	Pilot plant, HRT=12 days	Biogas production increased by 40%	[105]

Substrate	Pre-treatment	Anaerobic digestion condition	Result	Ref.
Mixed sludge	165 °C, 120 min	Bench test, 48 days	VSS destruction efficiency increased from 48% to 58%, and methane production increased by 13%	[102]
Primary sludge	170 °C, 30 min	Bench test, 24 days	Methane production increased by 78%	[34]
Municipal biowaste	175 °C, 60 min	Mesophilic anaerobic digester, HRT=20 days	Hydrolyzation rate increased by 10%	[103]
Mix sludge	165-180 °C, 30-60 min	WWTP, HRT=17 days	Electricity production increased by 20%	[107]

Ultrasonic treatment is a mechanical treatment, which can lead to sludge floc disintegration and microorganisms' lyses. In sludge treatment, low frequencies (20-40 kHz) are the most efficient [92]. A study conducted by Tiehm et al. [110] showed that ultrasonic treatment (41 kHz, 150 min) of activated sludge helped to increase VS removal from 21.5% to 33.7% in a semi-continuous anaerobic digestion, and the ultrasonic treatment (5000 kJ/kg TS) also be reported to increase biogas production by 36% in another study of semi-continuous anaerobic digestion [111]. Pérez-Elvira et al. [112] studied a continuous anaerobic digestion process with ultrasonic pretreated sludge (30 kWh/m^3 sludge), and 37% improvement of biogas production and 25% improvement of VS removal was reported. Ultrasonic treatment was also reported to be used in WWTPs. Neis et al. [113] reported 30% biogas production increase in a WWTP in Germany after 25% of the activated sludge treated by sonication.

Oxidation and alkali treatments are other widely used chemical treatments for anaerobic digestion. Ozone is one chemical used for oxidation. Ozonation can lead to partial sludge solubilisation, and yield increases with ozone dose. However, too high concentration of ozone will result in reduced apparent solubilisation due to oxidation of the solubilised components [114]. In batch tests, treatment with ozone (0.1 g O_3/g sludge) led to doubled methane production in 30 days treatment [114, 115]. H_2O_2 is another oxidizing agent. Rivero et al. [116] reported that H_2O_2 treatment (2 g H_2O_2/ g VSS) increased COD removal in a continuous sludge treatment reactor (HRT= 30 days) from 52% to more than 70%; additionally, the author found that longer H_2O_2 treatment time can achieve higher COD removal in the reactor. On the other hand, alkali treatment is reported to be effective in sludge solubilisation, which provides extremely high pH value of medium by adding alkaline substance. Alkaline treatment can destroy floc structures and cell walls by hydroxy anions. Additionally, high pH causes natural shape losing of proteins, saponification of lipid, and hydrolysis of RNA. Chemical degradation and ionization of the hydroxyl groups (–OH− → −O−) lead to extensive swelling and subsequent solubilization of gels in sludge [117]. However, high concentrations of Na^+ or K^+ may cause subsequent inhibition of anaerobic digestion [108]. Alkali treatment is normally combined with thermal treatment, and compared to thermal hydrolysis, alkali treatment temperature is lower. Valo et al. [101] used 1.65 g/L KOH to adjust pH of activated sludge to 10, and the activated sludge was treated under 130 °C for 60 min. Results showed that

alkali treated sludge led to 30% increment of biogas production in batch test and 75% increment in continuous sludge treatment reactor [101].

2.3.2.2 pH value

Most anaerobic processes operated best at near neutral pH, where methanogenic organisms can convert substrate, including acetic acid and hydrogen and carbon dioxide, to methane efficiently [87]. However, pH usually decreases as the result of excess production and accumulation of acidic or basic conversion products such as organic fatty acids or ammonia. Previous studies have reported that anaerobic reactors with pH less than 6 were often observed with decreased methane production and increased acid accumulation, which could result in complete failure of the reactor [118]. The explanation for reactor failure at lower pH is related to high concentrations of undissociated fatty acids, particularly propionic acid. The accumulation of fatty acids will lead to inhibition of the acetogens by reducing their ability to degrade the heavier acids into acetic acid [119]. Jain and Mattiasson [120] published work was showing that methane production at pH of 5.0, 4.5 and 4.0 was only 67%, 37%, and 34% of that achieved at neutral pH. However, they also found that methanogenic microorganisms could become acclimatized to the low pH values when system pH decreased over an extended period [120]. Given the rising costs of adjusting pH in WWTPs, researchers have focused on feasibility of sustaining methanogenesis at low pH in the laboratory scale anaerobic digester. Taconi et al. [119] operated a semi-continuous lab-scale reactor at pH ranging from 4.0-5.3, and the results showed that if the methanogens can sufficiently acclimated to acidic condition, comparable COD removal and methane production with neutral system can be achieved under acidic conditions. However, low pH value has not been reported to be feasible for pilot plants or WWTPs, since the low pH can cause failure of the anaerobic digestion system.

Some studies also revealed the effect of different pH on the shift of methanogenic pathways. There are two different pathways reported to transfer accumulated acetate to methane: one is acetoclastic methanogenesis (AM) operated by the acetotrophic methanogens, such as *Methanosaetaceae* and *Methanosarcinaceae*; the other is tandem reactions of syntrophic acetate oxidation (SAO) and the subsequent hydrogenotrophic methanogenesis (HM) by acetate-oxidizing bacteria and hydrogenotrophic methanogens [121]. Findings reported by Kotsyurbenko et al. [122] suggested that microbial populations and metabolic pathways have significantly different responses to pH,

turning from AM to HM with pH decreasing. In the study conducted by Hao et al. [123], acetoclastic methanogenesis was the primary pathway of methanogenesis, with the hydrogenotrophic methanogenesis accounting only 21-22% of total methane formation at pH of 6.0-6.5. Conversely, the dominant pathway changed to syntrophic acetate oxidation (SAO) and hydrogenotrophic methanogenesis (HM) at pH of 5.5, which accounts 51% of the methane formation.

2.3.2.3 Alkalinity

Alkalinity of sludge is derived from the breakdown of organics and is present primarily in the form of bicarbonates. Show et al. [124] concluded that the bicarbonate alkalinity ranges between 1000 and 5000 $CaCO_3$ mg/L during the typical anaerobic digestion process when pH ranges from 6.6 to 7.4 and the carbon dioxide proportion in the biogas is 30-40%. A few studies focused on the effect of alkalinity on the biogas production rate. Couderc et al. [125] found that the increased liquid phase alkalinity caused no increase of gas production in the anaerobic digestion of pit latrine sludge, and additional alkalinity in liquid phase may have a negative effect on the gas production rate. Another study conducted by Agdag and Sponza [126] showed that addition of $NaHCO_3$ with different concentrations (3 g/L.d and 6 g/L.d) has obvious impact on COD, volatile fatty acid concentration and accumulative methane production during 65 days anaerobic digestion. Addition of $NaHCO_3$ led to decreased COD and volatile fatty acid in leachate, and the accumulative methane production was increased by 58% and 90% by addition of 3 g/L.d and 6 g/L.d $NaHCO_3$, respectively [126]. Zhang and Jahng [127] tested three alkalis (NaOH, KOH and CaO) in the anaerobic digestion of piggery wastewater. The result showed that the methane production rate increased more than two folds when pH was adjusted to 9.5 - 10 by adding NaOH, KOH and CaO, however, cations of Na^+ and K^+ were stronger methanogenic activity inhibitors than Ca^{2+} in toxicity batch tests [127].

2.3.2.4 Temperature

Temperature is considered as one of the important determinants of the rate of anaerobic digestion, particularly the rates of hydrolysis and methane formation. Two optimal temperature ranges mesophilic (33 - 35 °C) and thermophilic (53 - 55°C), with decreased rates between these optima have often been cited [128, 129]. Mesophilic digesters are usually designed for SRT of 20-30 days, while thermophilic digester can be operated with lower SRT of 10-12 days [9]. The Table 2.7

summarizes the results of continuous anaerobic digestion under different temperature ranges. Different studies have tested variety of conditions under different temperature ranges. Most studies showed that thermophilic condition was more efficient than mesophilic condition. For example, Cecchi et al. [24] compared mesophlic (37 °C) and thermophilic (55 °C) anaerobic digestion with different SRTs and organic loading rates (OLRs). The results showed that, VS removal increased from 23% to 48%, and CH_4 production grew from 1.4 m^3/d to 2.5 m^3/d when digestion switch from mesophilc to thermophilic condition with similar SRT and OLR. However, when the thermophilic digester were overloaded with organic matters (OLR ≥9.2 kg VS/m^3.d), the VS removal and CH_4 production reduced remarkably compared to lower OLR level (6.9 kg VS/m^3.d). Similar results were also reported by Cavinato et al. [130] and Bolzonella et al.[23].

However, some studies showed similar performance of mesophilic and thermophilic process. A study conducted by Song et al. [131] found that there were little difference between mesophilic and thermophilic anaerobic digestion in terms of VS removal and CH_4 production. The authors also studied the performance of thermophilic and mesophilic temperature co-phase anaerobic digestion, which was consisted by an exchanging digesting sludge flow through mesophilic digester and a retention thermophilic digester. The results showed that co-phase anaerobic digestion achieved similar CH_4 production as single-stage mesophilic digestion, while VS removal (50.7-58.8%) was higher than single stage thermophilic or mesophilic digestion. Coelho et al. [25] even observed lower VS removal and gas production under thermophilic condition, the reason could be due to the overloaded organic loading rate [24]. Zinder et al. [129] tested different temperatures within the thermophilic range (50 °C and 58 °C), which indicated that higher temperature could benefit the CH_4 production slightly; however, hyper-thermophilic condition (70 °C) caused significant reduction (by approximately 30%) of gas production. Based on the different operation conditions and sludge sources, as well as different energy consumptions of thermophilic and mesophilic digestion, it is hard to draw a clear conclusion that thermophilic digestion is superior to mesophilic.

Table 2.7 Continuous anaerobic digestion of sludge under mesophilic and thermophilic conditions.

Temperature (°C)	SRT (d)	Reactor size (L)	Feed sludge characters			Organic loading rate (kg VS/m^{-3}.d)	VS removal (%)	Methane production (L CH_4/g VS_r*)	Reference
			TS (g/L)	VS (g/L)	COD (mg/L)				
35	27	150	51.4	34.8	71.7	1.37	53	0.4	[128]
	21	18	7.7	6	8.6	N.A	30	0.3	[98]
	20	12.2	N.A.	9.9	6.45 (soluble)	1.43	43	0.45	[131]
	20	1300	58.1	45	72	2.2	36	0.8	[23]
37	14	300	222	110	105	7.5	23	0.13	[24]
	22	380	23	22	21.6	1.2	N.A.	0.15	[130]
	20	0.8	51.4	36.1	60.3	1.8	37.4	0.09	[25]
50	10	3	350	200	N.A.	1.8	N.A.	0.2	[129]
53	15	22.5	30	15	N.A	N.A	56	0.4	[132]
55	27	150	51.4	34.8	71.7	0.96	53	0.24	[128]
	75	150	51.4	34.8	71.7	N.A.	71	0.09	[128]
	11	3000	164	81.6	128	6.9	43	0.27	[24]
	11	3000	230	103.5	130	9.2	34	0.17	[24]

Temperature (°C)	SRT (d)	Reactor size (L)	Feed sludge characters			Organic loading rate (kg VS/m^{-3}.d)	VS removal (%)	Methane production (L CH_4/g VS_r*)	Reference
			TS (g/L)	VS (g/L)	COD (mg/L)				
	7	3000	224	105	183	13.5	37	0.18	[24]
	10	5	N.A.	9.9	6.45 (soluble)	2.9	46	0.41	[131]
	22	380	23	22	21.6	1.66	N.A.	0.49	[130]
	20	0.8	51.4	36.1	60.3	24.1	16	0.51	[25]
	20	1300	58.1	45	72	2.2	48	0.9	[23]
58	10	3	350	200	N.A.	2.7	N.A.	0.26	[129]

* Note: VS removed

2.3.2.5 Nutrients

The nutrient requirement for anaerobic digestion includes some fundamental macronutrients such as carbon, nitrogen, phosphorus and sulphur and some micronutrients such as Ca, Mg and Fe. Nitrogen is the major essential nutrient during digestion, and the Figure 2.4 demonstrates the nitrogen conversion during anaerobic digestion. During anaerobic digestion, complex organic N compounds are mineralized to NH^{4+}-N. A part of the NH^{4+}-N is used by microorganisms for growth. Further processes are formation of struvite and ammonium carbonate; traces are volatilized in the biogas stream [133].

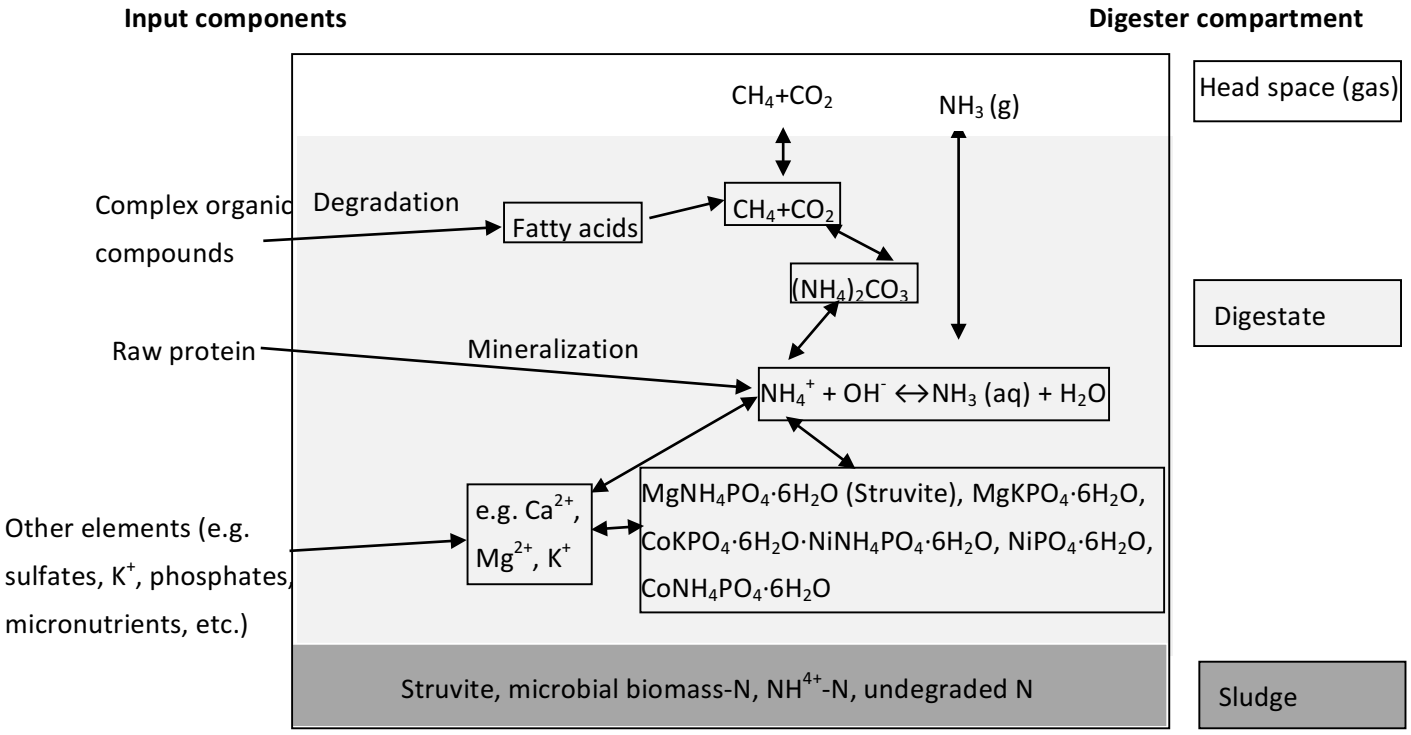


Figure 2.4 Nitrogen conversion during anaerobic digestion [133].

Generally, C:N ratio of substrate is frequently utilized to describe the nutrient requirement. Previous studies have reported that C:N ratio of 25-30:1would be ideal for microorganisms during co-digestion [134, 135]. The C:N ratio of substrate has been reported to play an important role in the acidification efficiency of the substrate [136]. Moreover, C:N ratio can affect the methane production of anaerobic digestion. Wang et al. [137] studied anaerobic digestion of dairy manure and chicken manure, which

showed that C:N ratio of 27.1 is optimized for maximum methane potential. However, wastewater sludge has a much lower C:N ratio of 9:1 than ideal level [138]. Therefore, researchers have reported the improved methane production of anaerobic digestion by adding agriculture waste or municipal solid wastes. Gómez et al. [139] conducted a lab-scale digester to treat the mixture of primary sludge (22%) and the fruit and vegetable fraction of the municipal solid wastes (78%), and the results showed that sludge mixture produced more biogas than primary sludge only. Romano and Zhang [140] studied the co-digestion of onion juice and wastewater sludge using an anaerobic mixed biofilm reactor, which showed that C:N of 15 and OLR of 3.6 g VS/L/d were recommended for treating mixed sludge. Co-digestion process of waste activated sludge with the organic fraction of municipal solid wastes were also studied in WWTPs [141]. Addition of organic waste (organic fraction of municipal solid waste) was used in WWTPs in Italy. The organic waste (3 tons per day) and waste activated sludge (20 tons per day) were mixed and fed to digester, showing approximately 50% increment of biogas production [141]. Cattle manure was also studied as additional organic waste source for sludge digestion. Shilton et al. [142] reported a batch test of primary sludge (100 mL/d) and cow manure (50 mL/d) co-digestion, and the results showed twice higher biogas production than primary sludge digestion. As the by-product of biodiesel production, glycerol has been reported to benefit anaerobic digestion of sewage sludge [143, 144]. 1% glycerol addition into a lab-scale sludge digester (1 L) was reported by Fountoulakis et al. [143], which showed a significant increase of methane production from 1106 mL to 2353 mL. Furthermore, the effect of glycerol addition in a pilot-scale digester (1300 L) was studied by Razaviarani et al. [144]. The authors observed 65% and 83% growth of biogas production potential and methane production potential, respectively, by 1.1% biodiesel waste glycerol addition. It is notable that high proportion of glycerol will lead to methane production decrease. As Fountoulakis et al. [143] reported addition of 3% of glycerol resulted in volatile fatty acid accumulation and process instability of the digester. On the other hand, COD: N ratio was also used to evaluate nutrient

requirement, and the high and low substrate COD:N ratios were 400:7 and 1000:7, respectively [87].

Sulphur is another essential nutrient for anaerobic digestion. The COD: SO_4^{2-} ratio is regarded as the determinant for the syntrophy and competition between different groups of bacteria. When the COD: SO_4^{2-} ratio is 2, the methane producing bacteria prevails over the sulphur reducing bacteria in acetate degradation, while the sulphur reducing bacteria are more dominant in H_2 utilization [145]. However, a COD: SO_4^{2-} ratio of 4 between 16 will lead to the methane producing bacteria dominating acetate degradation and hydrogen utilization. The researchers reported the COD: SO_4^{2-} ratio was ranging from 4.98 to 6.59 in a pilot anaerobic digester [144], where methanogen dominates the acetate degradation and hydrogen utilization

Additionally, there are other trace metal elements essential for methanogenesis of anaerobic digestion, such as Fe, Ni, Ca, Na and Xe. These elements are implicated in the enzyme system of acetogenic and methanogenic bacteria [87]. For example, cobalt (Co) was reported to be implicated with methyltransterase and B12-enzymes of methanogens and acetogens, and Ni was involved with formation of methyl-CoM-reductase of methanogens [146]. A research conducted by Pobeheim et al. [147] showed that the increase in Ni^{2+} concentration from 17 μM to 34 μM led to 20% growth of methane production in anaerobic digestion of maize and sludge. The trace metal elements also play important role on the microbial respiration processes with an electron transfer bound to cell wall or extracellular electron acceptors [146]. On the other hand, all metals are potentially inhibiting microbial activity, which will be detailed discussed in next section.

2.3.2.6 Toxicity and inhibition

The methanogenic processes can be inhibited by several toxic substrates in a variety of circumstances. Volatile fatty acid, for example, is one of the inhibiters of anaerobic digestion. Volatile fatty acids, including long chain fatty acid like stearic, oleic, linoleic, and short chain fatty acid like acetic, butyric, propionic, valeric and iso-valeric acids,

are generated during hydrolysis process [148]. The methanogenic microbial growth could be restricted in the presence of excessive volatile fatty acids [87]. Due to the different wastes treated, the volatile acid concentration during anaerobic digestion could vary between 100 and 5000 mg/L [148]. The overall inhibitory effect of the volatile fatty acids is related to the pH established by the prevailing buffer system. Long chain fatty acids are known to inhibit the methanogenic activity even at low concentrations. The inhibitory effect was initially attributed to toxicity resulting from cell damage and it is known to affect both syntrophic acetogens and methanogen [149]. Angelidaki and Ahring [150] observed that 200 mg/L oleate or 500 mg/L stearate inhibited the thermophilic anaerobic digestion of cattle manure, which decreased the methane production by 60%; and 500 mg/L oleate or 1000 mg/L stearate permanently inhibited the growth of bacteria with no methane production. Lalman and Bagley [151] reported that, inhibition effect of 30 mg/L linoleic acid on aceticlastic methanogensis was observed in an anaerobic system treating wastewater with vegetable oils. Some researchers also reported the inhibitory effect of long chain fatty acids on anaerobic digestion by modelling. For example, Lokshina et al. [152] clarified the inhabitation effect of long chain fatty acid of 5.8 mM on the anaerobic digestion of solid poultry slaughterhouse wastes by applying the <METHANE> simulation model. However, further studies have demonstrated that long chain fatty acids inhibition is reversible and the microorganisms are able to efficiently methanise the accumulated fatty acid after a lag-phase [112]. In the continuous-flow digester, even the methanosarcina methanothrix were inhibited by accumulated fatty acids, the growth of hydrogenotrophic methanogens can lower the hydrogen concentration, which, in turn, can remove hydrolysis inhibition [112]. Palatsi et al. [153] studied several strategies to recover inhibited anaerobic digestion by 4 g/L long chain fatty acid addition. The results showed that self-recovery process was the strategy resulting in slowest recovery time of 40 days, while addition of fresh manure (1 g VS/L.d) and bentonite powder (5 g VS/L.d) led to short recover time of 9 days and 7 days, respectively. Thus, increasing the ratio of biomass/long chain fatty acids, or the addition of adsorbents, were the best

strategies to recover inhibited thermophilic manure reactors. On the other hand, short chain fatty acids, like acetic, butyric, propionic acid, are most common acid produced during the anaerobic degradation of organic matters. It is suggested that high concentrations of acetate have been shown to retard the primary breakdown of organic material, but did not affect the activity of the methane bacteria [148]. Propionic acid is the ultimate fatty acid prior to methanation, which was reported to be specifically inhibitory to the process. The composition and proportion of acids may vary during acid toxicity; therefore, the ratios of (propionic + butyric acids) / acetic acid were critical to methane production. Stafford [148] reported that the best ratio of (propionic + butyric acids) / acetic acid was less than 80:1; above which, the inhibition of biogas production occurred.

Ammonia, produced by biodegradation of proteins and urea, is regarded as one of the inhibitors in anaerobic process. The mechanisms for ammonia inhibition have been proposed as a change in the intracellular pH, increase of maintenance energy requirement, and inhibition of a specific enzyme reaction [154]. Among the anaerobic microorganisms, methanogens are the most likely to be inhibited due to ammonia. A study showed that methanogenic population lost 56.5% of its activity when ammonia concentration increase to 4051 – 5734 mg NH_3-N/L [155]. It is believed that ammonia is an essential nutrient for anaerobic microorganism with concentration below 200 mg/L; however, methane production has been reported to be reduced by 50% with the total NH_3-N concentration ranging from 1700 to 14000 mg/L in literature [156]. Sulphide is also reported to cause inhabitation for anaerobic digestion. Parkin et al. [157] reported the inhibitory sulphide level ranges were 100-800 mg/L dissolved sulphide or 50-400 mg/L undissociated H_2S.

Light metal ions, such as Na^+, K^+, Ca^{2+} and Mg^{2+} are usually present in the sludge system, which are essential for microorganisms. Their toxicity has been reported in previous studies (Table 2.8). Moreover, the heavy metals, including Cr^{6+}, Cr^{3+}, Cu^{2+}, Ni^{2+} and Zn^{2+}, were identified to be inhibitory for anaerobic process (Table 2.8). It is

suggested that, the inhibitory effect was related to the physical-chemical forms of heavy metal, and only metals in soluble, free form are toxic to the microorganisms. It is interesting to note that toxicity decreased in the of order of Cu>Zn>Ni, Which might be explained by the fact that Zn and Ni are components of several enzymes in anaerobic microorganisms [156]. Oleszkiewicz and Sharma [158] summarised several method for detoxification of heavy metals, including precipitation, sorption and chelation by organic and inorganic ligands. Among them, precipitation with sulphide was the most widely used method.

Table 2.8 Concentration of metal ions reported to be inhibitory to anaerobic microorganisms [87].

Metal ion	Concentration (mg/L)	
	Moderate inhibition	Strong inhibition
Na^+	3500-5000	8000
K^+	2500-4500	12000
Ca^{2+}	2500-4500	8000
Mg^{2+}	1000-1500	3000
Cu^{2+}	N.A.	0.5 (soluble)
Cr^{6+}	N.A.	3 (soluble)
Cr^{3+}	N.A.	180-420 (soluble)
Zn^{2+}	N.A.	1 (soluble)
Ni^{2+}	N.A.	2 (soluble)

2.3.3 Performance indicators of anaerobic digestion

Operational experiences of anaerobic treatment process have revealed that effective methods of process monitoring and control are of great importance in terms of:

1) the achievement of a consistently high degree of waste stabilization
2) high conversion of waste to methane. Monitoring and associated process control can be implemented in either slurry phase or in the gas phase, involving measurement of pH, alkalinity, total and individual volatile acid, COD, gas analysis for methane and carbon dioxide.

2.3.3.1 pH, alkalinity and volatile acids

The pH value of anaerobic process is of great importance for anaerobic microorganisms, especially methanogenic bacteria, which require an optimum between 6.5 to 7.2 [28, 29]. Fermentation microorganisms, which can function between 4.0 and 8.0 with different products at different pH values, are less sensitive. At low pH, the main products are acetic and butyric acid, while products tend to be acetic and propionic acid at pH of 8.0 [29]. The change in pH can be an indicator for the anaerobic process, and the cause of process imbalance. The accumulation of volatile fatty acids can cause the reduction of pH, which also produce alkalinity in the form of carbon dioxide, ammonia and bicarbonate [28]. However, pH cannot be used as an indicator for process imbalance in a well buffered system, because the change of pH from volatile fatty acid accumulation is too small [29].

Alkalinity is a better indicator than pH for indicating volatile fatty acid accumulation during anaerobic digestion, since the alkalinity will be directly consumed by increased volatile acid. Total alkalinity is usually measured by titration of samples to pH 4.3. Switzenbaum et al. [30] suggested that the ratio of volatile fatty acid : total alkalinity between 0.1- 0.35 would be an indicator for healthy digester. Apart from pH and alkalinity, the concentration of volatile fatty acid is also a popular parameter for sludge digestion monitoring. The volatile fatty acid can be measured on-line, by estimation with pH [159] or electrical conductivity of the digester [160].

2.3.3.2 Gas phase monitoring

Gas phase monitoring is frequently applied to assess the efficiency and state of anaerobic processes stabilization. Biogas produced from anaerobic digester contains about 60-70% methane, 30-40% carbon dioxide, and trace amounts of nitrogen, hydrogen, hydrogen sulphide and water vapour [28]. The specific gas production was reported as 0.75-1.12 m^3/kg $VS_{removed}$, or 0.5-0.75 m^3/kg $VS_{loading}$ [28]. The biogas production rate, especially the methane yield can act as an indicator of the metabolic status of the digester. The reduction of specific methane production is the sign of imbalanced accumulation of soluble acid product in the liquid phase during the continuous-flow system [30]. Other trace gas in the gas phase, such as hydrogen and carbon monoxide, can also indicate the status of an anaerobic system. Some studies have reported that the concentration of H_2 and CO in gas phase had a clear relation with the volatile fatty acid accumulation in liquid phase [161, 162]. Castellano et al. [162] reported the H_2 concentration in biogas presented the classification of different steady states, and Hickey [161] found a strong correlation between the CO_2 concentration in the gas phase and the acetate concentration in the liquid phase.

2.3.3.3 Other parameters of anaerobic process

There are other parameters could be monitored during anaerobic process to achieve an efficient anaerobic process, including COD removal, heavy metals etc.

COD removal of anaerobic process mainly depended on the organic loading rates of the system, rather than the HRT or the COD level alone. Several studies reported the COD removal varied between 40 – 60% when the organic loading rate ranging from 2.2 to 7 kg VS/m^3.d [23-25]; however, some studies reported higher COD removal up to 80 – 90% when organic loading rates ranging from 0.8-4 kg COD/m^3.d [26, 27]. It is commonly reported that increasing of organic loading rate could decrease the COD removal in the digester [24, 27, 163].

The occurrence of heavy metal in wastewater sludge has been widely reported [87, 156, 158]. Heavy metals are non-biodegradable and occur in various forms in sewage sludge and its anaerobic process, including: (1) precipitation as sulphide, carbonate and

hydroxides, (2) sorption to the solid fraction, either biomass or inert particulate matter and (3) formation of complexes in solution with intermediates and product compounds produced during digestion [156]. Therefore, it is difficult to distinguish a particular heavy metal and its concentration in the sludge. Several studies have observed that heavy metals could concentrate during the anaerobic digestion process [28, 164-166], due to the weight loss as the result of organic matter decomposition, biogas releasing and other processes [28]. Dong et al. [165] and Cai et al. [166] observed approximately 50% increase of Cu, Zn, Pb and Ni during anaerobic process. However, Selling et al. [167] conducted a two-stage anaerobic digestion system, which was able to remove up to 70% of Ni, 40% of Zn. The authors explained that heavy metals can be transferred to the leachate by hydrolysis/acidification and liquefaction of the substrate, then heavy metals was removed by adsorption of macroporous polyacrylamide monolith columns in the second stage [167].

2.3.4 Advantages and disadvantages of anaerobic sludge digestion

As a widely-used sludge reduction technology, anaerobic sludge digestion aim to transfer wastewater sludge to innocuous and easily dewatered substance, as well as to reduce the quantity of solids and volume of sludge for disposal. The advantages of anaerobic digestion compared to other methods include:

1) A usable energy source (methane) can be generated during anaerobic digestion. Although the anaerobic digesting plant requires additional energy for mixing, the process is a net energy producer at most treatment facilities. The surplus energy also can be used to heat building, to generate electricity to drive aeration blowers, sewage pumps, etc.
2) Anaerobic digestion can achieve 25-45% reduction of the feed sludge solid [87], which can result in the reduction in the cost of sludge disposal.
3) Digested sludge can be used as fertilizer for agriculture purpose [168]. The anaerobically digested sludge contains nitrogen and phosphorus and other nutrients that can improve the fertility and texture of soil.

4) Pathogens can be inactivated during anaerobic digestion process [87].

Despite these advantages, anaerobic sludge digestion also entails some inevitable disadvantages:

1) The capital costs are high. Large, covered tanks with pumps for feeding and circulating sludge, heat exchangers are required [87].
2) The reaction rate is slow, leading to longer retention times (more than ten days) to develop and maintain a population of methane-producing bacteria [28].
3) The presence of other biogas constituents such as CO_2, H_2S, moisture and volatile siloxanes, can cause serious damage in the generator and boiler [28].
4) Heavy metals and some organic contaminants are non-degradable during anaerobic digestion, which lead the increased concentrations in the residual sludge. These toxic compounds in the digested sludge could be transferred to the food chain via farming [168].

2.4 Pollutants management for land application of sludge

Treated sewage sludge, also called biosolid, is the major by-product of the wastewater treatment process. Due to the increasing concern for environmental pollution of oceans and waterways from sludge disposal, land application has become a beneficial approach for wastewater sludge disposal. Biosolids have a high nutrient content and can condition solid to improve its structure and water retention qualities, therefore it is usually applied for agriculture and forestry land reclamation. Moreover, composted sludge can be used to improve the soil's physical properties such as water holding capacity and soil structure in some areas of US [168].

Australia currently produces approximately 300,000 dry tonnes of biosolids annually [50]. Approximately 55% is applied to agricultural land and around 30% is disposed of in landfill or stockpiled. The remaining 15% is used in composting, forestry, and land rehabilitation or incinerated. According to the "Environmental guidelines: Use and disposal of biosolids products" published by EPA New South Wales, the biosolids can

be classified in terms of the manner biosolids products may be used: unrestricted use, restricted use, or not suitable for use [169]. To identity the classification of a biosolids products, it is necessary to determine both its contaminant grade and the stabilisation grade. The contaminants acceptance concentration thresholds were used to determine the contamination grand to A, B, C, D or E with Grade E being the lowest grade. Additionally, the stabilisation grade (A, B and C) was determined by the process and microbiological verification that the process is performing effectively. Anaerobic digestion was degraded as stabilisation grade B. The classification of biosolids production and their land use in NSW are summarised in Table 2.9 [169].

Table 2.9 Classification of biosolids products

Biosolids classification	Allowable land application Use	Minimum quality grades	
		Contaminant grade	Stabilisation grade
Unrestricted use	Home lawns and gardens; Public contact sites; Urban landscaping; Agriculture; Forestry Soil and site rehabilitation; Landfill disposal; Surface land disposal.	A	A
Restricted use 1	Public contact sites; Urban landscaping; Agriculture; Forestry; Soil and site rehabilitation; Landfill disposal; Surface land disposal.	B	A
Restricted use 2	Agriculture; Forestry; Soil and site rehabilitation; Landfill disposal; Surface land disposal	C	B
Restricted use 3	Forestry; Soil and site rehabilitation; Landfill disposal.	D	B
Not suitable for use	Surface land disposal; Landfill disposal; Surface land disposal.	E	C

Similarly, a guideline for biosolids land application was issued by EPA Victoria [170]. Classification of biosolids is based on two independent factors, namely the contaminant concentrations in the biosolids and the microbiological quality post treatment. The classifications within these factors are:

(i) Contaminant Grade (C1 or C2) based on biosolids contaminant concentrations; and

(ii) Treatment Grade (T1, T2, T3) based on the treatment technology utilised, microbiological criteria and measures used to inhibit bacterial regrowth, vector attraction (such as insects or vermin) and odour. Accordingly, biosolids was permitted for restricted uses for agricultural or non-agricultural use.

It is important to note that several factors, such as crop cultivation patterns, soil conditions, weather conditions and fertilizer requirement, must be considered when sludge is used for agricultural purpose [168]. However, pollutants which are resistant to sludge treatment could lead problem of community health and environmental justice [51, 52].

2.4.1 Sludge odour

There have been a few research evaluating health and quality of life near sludge land application sites, and the foul odour has been frequently reported to be the major concern [52, 53, 171]. Some residents associate physical symptoms such as respiratory distress, headaches, and skin rashes with the odour components caused by land application of sewage sludge [52, 53]. Thus, necessary solutions for managing and minimizing odour need to be implemented during the sludge treatment and land application. Odours from wastewater sludge arise as a result of bacterial activity, which are normally composed of hydrogen sulphide, ammonia, amines, mercaptans, organic acid and skatoles [55]. Table 2.10 lists the odour threshold values. However, hydrogen sulphide is usually used as indicator of odour level.

Table 2.10 Odour threshold values [54].

Compound	Threshold valve (ppm)
Acetic acid	1.0000
Ammonia	46.8000
Butyric acid	0.0010
Chlorine	0.3140
Ethyl mercaptan	0.0010
Methyl mercaptan	0.0021
Hydrogen sulphide	0.0005
Skatole	0.2200

Currently, there are five odour treatment methods available for sludge odour control, which are wet scrubbing, activated carbon absorption, activated sludge scrubbing, bio-scrubbing and biofiltration [56]. The following Table 2.11 listed the odour removal efficiencies by different methods. The observations made by Lang and Jager [56] indicated that high odour removal was achieved in several US municipal sludge treatment plants by biofiltration, wet scrubbing and activated carbon. Another option includes avoiding the emission by enclosing parts of the process in order to capture and treat the gas [172]. Moreover, bioscrubber can also be applied to minimize odour gases by approximately 70% [172].

Table 2.11 Odour removal efficiency by different methods in USA (summarised from [56]).

Method	Inlet odour (D/T)	Removal (%)	Location
Biofiltration	222-650	54-97	Westborugh
	400	90-99	Gainesville
Wet scrubbing	180	80	Lancaster
	125-212	80	Schenectady
Activated carbon	34-73	75	Westborugh

2.4.2 Risks associated with heavy metal

The fertilizer value of biosolids has been known for a very long time; however, agricultural application of wasted sludge can also pose the risk to food security. Toxic elements, such as heavy metals, are persistent during the sludge treatment process; and such elements could accumulate in agriculture soil due to long-term use [51, 173]. Once accumulated, heavy metals are highly persistent in the topsoil and can cause potential problems or elevated transfer to the food chain [51, 174], which may pose a serious risk to human health [175]. The phytotoxicity of sewage sludge derived heavy metals depends on various factors such as nature and amount of heavy metals, degree of metal association in sewage sludge, soil and plant characteristics etc. [176].

Heavy metal like Cu, Zn, Mn, Ni, Cd etc. were widely reported in the research study the plant accumulation and bioavailability of heavy metal when soil was fertilised by sewage sludge [51, 173-175]. In the study conducted by Kidd et al. [174], the concentrations of Cu and Zu were found to be increased in the soil fractions in soils with a history of sewage sludge application, and the increased concentration of Cu and Zn were also observed in the root tissue and aerial parts of corn crop (*Zea mays*) and wild plant (*Cistus ladanifer*). Furthermore, another study also revealed that Cd, Cr and Pb concentrations in anaerobically digested sludge amended soil (90 t/ha dry weight)

were similar to control plots, whereas radish tops (*Raphanus sativus*), bean leaves (*Phaseolus vulgaris*) and corn leaves (*Zea mays*) showed higher concentrations of these elements under two of the sewage sludge treatments [177]. Cd generally tends to accumulate in leaves, and therefore is more risky especially for leafy vegetables grown on contaminated soils and the consumption of such plants might pose a serious risk to human health [178]. Lo´pez-Mosquera et al. [173] investigated 12 grassland plots fertilised over a 1 – 4 year period with dairy sludge. The results showed that there were no significant differences in soil heavy metal concentrations between the sludge-amended plots and the control plots, expect Cr. However, several significant metal-metal correlations (Ni–Zn, Ni–Cr, Cu–Cr, Zn–Cr, and Pb–Cd) were observed in both the sludge and sludge-amended soils but not non-sludge-amended soils. These findings suggest the dairy sludge is a source of heavy metals for the soil, and long term sludge use will eventually lead to a build-up of heavy metals [173].

2.4.3 Trace organic contaminants occurrence and removal by anaerobic digestion

Trace organic contaminants (TrOCs) means organic substances whose toxic persistent and bioaccumulative properties may have a negative effect on the environment and/or organisms. They are present in many products that we consume daily (drugs, cosmetics, phytosanitary products, insecticides, etc.), at the home or in industry. In recent years, with the significant advancement of analytical methods, TrOCs have been frequently detected in wastewater-impacted water sources all over the world [179-182].

As consumed from household, TrOCs include pesticides, industrial chemicals, components of consumer products, pharmaceuticals and personal care products, hormones etc. are regularly released into municipal sewage by anthropogenic activities [183]. Due to the lipophilicity of TrOCs, the compounds can transfer to the sewage sludge during the wastewater treatment processes (primary and secondary clarification) [57-59]. As a result, TrOCs in municipal wastewater sludge can be detected in both

aqueous phase (several μg/L or more) and solid phase (several μg/kg dry weight or more). Antibiotics and pharmaceutically active compounds were amongst the most investigated TrOCs in digested sludge. Trimethoprim, sulfamethoxazole, ciprofloxacin and doxycycline were notable antibiotics detected at the low mg/kg dry weight range in digested sludge from Swedish WWTPs [184, 185]. Ciprofloxacin and diphenhydramine were also detected in more than 80 sludge samples across the USA [186]. In Japan, Narumiya et al. [61] reported the occurrence of 45 TrOCs in digested sludge. Concentrations of several compounds (e.g. ofloxacin, triclosan and triclocarban) exceeded 1 mg/kg dry sludge [61]. Several personal care products including triclosan and triclocarban have also been reported to accumulate in the digested sludge to a high concentration after anaerobic digestion [187, 188].

It must be mentioned that significant differences can be observed for even the same compounds due to different sampling locations and time. The factors affecting TrOCs accumulation in sludge includes the compounds concentration in influent wastewater, physico-chemical properties of the compounds (molecular weight, hydrophobicity, water solubility, pKa, functional groups), the sludge characteristics (pH, organic matter, cations' concentration) and the operational parameters (SRT, temperature) [58, 189]. The value of pH has been reported to affect the sorption of TrOC during sludge treatment. In a study conducted by Urase and Kikuta [190], it was found that that TrOCs with carboxylic acid groups (e.g. fenoprop) absorbed more onto the sludge when pH reducing from 7 to 5 because the un-dissociated and neutral form of the compounds predominated at lower pH. On the other hand, some compounds could be desorbed when pH is changing. For example, bisphenol A was found to be desorbed from activated sludge when pH increasing from 7 to 9-12 in batch test [191]. The extracellular polymeric substances (EPS) were also reported to influent the TrOC sorption. Increased EPS concentration could enhance the sludge hydrophobicity, and consequently increase the affinity towards TrOCs [192, 193]. Additionally, temperature could affect the sorption of some compounds, for examples, 17α-ethinylestradiol was

absorbed more on the anaerobic sludge at 10 °C than that 30 °C [194]. The reason could be that the sorption of TrOCs on sludge is an enthalpy-driven process, and temperature can affect the electrostatic interactions between compounds and sludge [195].

Although TrOCs have been commonly found in municipal sewage at very low concentrations [196], some of these TrOCs have the potential to cause chronic disorders in animals and humans at a sufficient concentration. Several countries have already imposed controls on certain TrOCs such as nonylphenol (NP) and nonylphenol ethoxylates (NPEs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and dibenzo-p-furans (PCDD/Fs). However, a unified directive to address TrOCs in digested sludge has not yet been developed [60]. Persistent TrOCs have the potential to bioaccumulate during land application and, if left unchecked, may impose adverse risk to humans and the ecosystem. Hence, the removal of TrOC during sludge treatment has drawn a lot interest from researchers. Many of the previous studies concerning anaerobic treatment have focused mostly on the removal of TrOC from the aqueous (water) phase. Thus, the results are not applicable to anaerobic digestion of sewage sludge. Indeed, results from some studies [61-65, 197] examining the removal of TrOCs from both aqueous and solid phases by anaerobic digestion show that the overall removal efficiency could be much lower compared to the value estimated ignoring the residual in the solids phase. Several compounds were found well removed from lab-scale anaerobic digesters, such as trimethoprim [61, 62], citalopram [62], sulfamethoxazole [61, 63], caffeine [61], naproxen [64], diclofenac [64], estrone [64, 65], 17α-ethinylestradiol [64, 65]. However, other compounds like fluoxetine [61, 63], carbamazepine [61, 62] and iopromide [64] were recalcitrant to anaerobic digestion.

It is important to note that most previous studies involved the spiking (artificial addition) of TrOCs to the feed sludge at elevated concentrations. For examples, Malmborg and Magner [62] studied the fate of 14 different TrOCs during the anaerobic digestion by spiking each compound at 50 mg/L into the sludge. They showed that several

compounds (e.g. trimethoprim, citalopram, and furosemide) were well removed by anaerobic digestion. However, several other compounds including fluoxetine and carbamazepine were persistent to anaerobic digestion. Similar results were reported by Carballa et al. [63] who added TrOCs to feed sludge at concentrations between 4 and 400 μg/L. It is important to note that the TrOCs concentration in the feed sludge was much higher than real wastewater sludge due to the artificial spiking; the results may not be applicable for full-scale digesters in WWTPs. Narumiya et al. [61] was amongst very few studies that monitored the environmental concentrations of TrOCs in the feed sludge. Narumiya et al. [61] showed that 4 out of 26 compounds, namely, sulfamethoxazole, trimethoprim, caffeine and acetaminophen detected in the thickened sludge were well removed by anaerobic digestion while most of the remaining compounds were not significantly removed. A few studies reported the TrOCs (estrogen) mass balance during anaerobic digestion in WWTPs. Marti and Batista [198] observed higher estrogen concentration (combination of estrone, estradiol and estriol) in sludge aqueous phase (581 ng/L) than the feed primary sludge (47.8 ng/g dry sludge) after anaerobic digestion in a full scale WWTP in USA. Similar results were found in Germany [199] and Canada [200]. Andersen et al. [199] found an increased estrogen concentration in the anaerobic digester effluent (67.1 ng/L estrone) compared to the aqueous phase of activated sludge (1.4 ng/L estrone). Lorenzen et al. [200] measured significantly higher estrogen in sludge after anaerobic treatment (1233 ng/g) compared to aerobic treatment (11.2 ng/g) for 19 WWTPs in Canada.

2.5 Recuperative thickening

Recuperative thickening is a modified anaerobic digestion process which was first demonstrated by Torpey and Melbinger in 1967 [35]. Compared to conventional anaerobic digestion, an additional thickening process is added to separate solid and digestate, and thicken sludge is returned to the digester with feed sludge (primary sludge and/or waste activated sludge) (Figure 2.5). As a result, recuperative thickening allows to extent SRT from HRT when digested solid is returned to the digester.

Recuperative thickening has been reported to improve anaerobic digester performance, biogas production in full scale plant application.

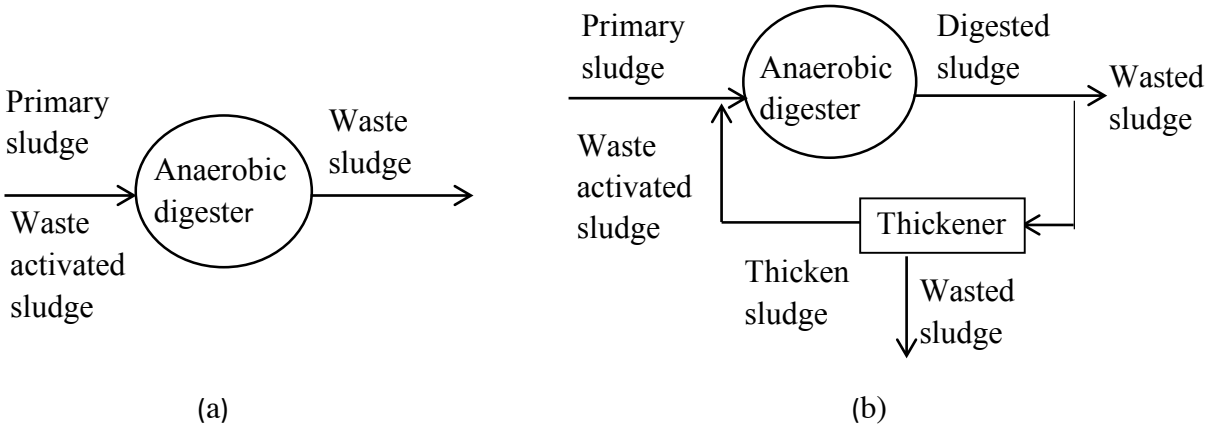


Figure 2.5 (a) Conventional anaerobic digestion and (b) anaerobic digestion with recuperative thickening.

2.5.1 Sludge thickening and consolidation

Sludge thickening and consolidation is an essential and economical part for sludge treatment process, especially anaerobic digestion with recuperative thickening. Thickening can effectively reduce the sludge volume and increase the sludge solids concentration, which allows reducing anaerobic digester volume. The following section will discuss the methods for sludge thickening and consolidation.

2.5.1.1 Gravitational thickening

Gravitational thicker is a conventional thickening process. As shown in Figure 2.6, the feed sludge entering in the middle is distributed radially, and the thickened sludge is collected as underflow in the sludge sump [85].

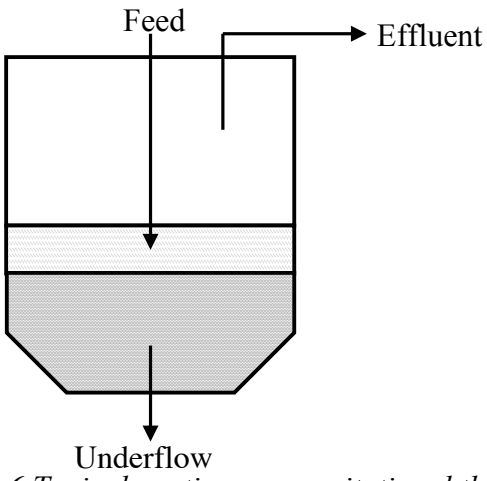


Figure 2.6 Typical continuous gravitational thickener.

The settling of sludge is slow due to its low specific gravity; therefore, the solids flux (kg solid /hr/m^2) can be used as an important criterion to design the gravitational thickener. Literature reported the solids flux of activated sludge and raw primary sludge are ranging from 0.8 - 1.0 kg/hr/m^2 and 1.6-3.8 kg/hr/m^2 [85]. The solids flux can be used to design gravitational thickener by calculating the required surface area by dividing the anticipated solids feed by the flux. In additional, gravitational thickeners are typically covered on-site to control the significant odours from the treatment. Gravity belt thickener is another popular method for thickening sludge. Solids are concentrated as free water drains by gravity through a porous horizontal belt in gravity belt thickening. Polymers are usually added for sludge chemical conditioning in gravity belt thickening process.

2.5.1.2 Dissolved air flotation thickener

In dissolved air flotation thickening (DAFT), microscopic air bubbles are attached to the sludge particles, reducing their specific gravity to less than that of water, which results in the floatation of the particles to the surface of the thickener tank for removal by a skimming mechanism [201]. It is important to note that DAFT is often used to thicken light and fluffy sludge, like activated sludge, which can be thicken by flotation quite readily; while raw sludge, for example, is more easily settled by gravity since it is heavy and tend to settle [85]. In sludge treatment plants, polymer is frequently added

into the recycle sludge flow to increase the solids loading rates and solids capture in DAFT. Normally, DAFT allows the solid loading rate of 1.9 – 5 kg/hr.m^2, and addition of polymer increase the solid loading rate up to 10 kg/hr.m^2 [201]. On the other hand, the sludge volume index (SVI) can be used as an indicator for solid characteristic during DAFT. An SVI of 125 for less is the optimum sludge for DAFT [201].

2.5.1.3 Rotary drum thickener

The rotary drum thickener consists of a rotating drum to which the conditioned sludge is applied. In the operation of rotary drum thickener, the sludge flows through the surface of the drum, and solid is transported by the spiral screw along the drum and thickened sludge discharged out the end of the drum [202]. Polymer conditioning is also necessary for improving flocculation of sludge and the thickening performance of rotary drum thickener. The variable speed drive unit rotates the drum at 5 - 20 rpm approximately [201], and can achieve the thickened solids concentration of 4 - 9% solids [202]. Gabb et al. [203] reported that rotary drum thickeners achieved cake solids concentration of 14% in one Oakland WWTP, and cationic emulsion polymers are proved to be the most successful polymer additives. The authors also observed that rotary drum consumed less polymers and achieved higher cake solids concentration compared to gravity belt thickener in pilot experiment [203]. However, rotary drum thickeners are less efficient than gravity belt thickeners in terms of unit capacity [202, 203].

2.5.1.4 Centrifuges

Centrifugal thickeners use centrifugal force to separate solids from liquid, which can typically achieve solid concentration of 5 - 8% [204]. The most common centrifuge technology is the solid bowl conveyor. The solid bowl rotated on its longitudinal axis is the typical configuration of centrifugal thickener. The feed is introduced by a central feed pipe, which sprays the sludge into the machine. It has been recognised that the introduction of the feed is an important component of successful continuous centrifugation. The feed tends to splash into the rapidly rotating sludge, which may

destroy some of the separation that has already occurred. Therefore, it is necessary to accelerate the feed in a very short time, and typical hydraulic residence time is a solid bowl centrifuge is about 20 seconds [205]. Furthermore, the hydraulic feed rate to the centrifuge also affects the solid capture rate, and increase the hydraulic load will decrease the solid capture [201]. Therefore, the performance of centrifuge thickener can be measured by thickened solids and solids capture, which can be adjusted to desired values by modifying feed flow rate, bowl and conveyor differential speed, polymer addition, and pool depth [201]. It is general recognized that, centrifuge thickeners cost more than gravity belt thickener or rotary drum thickeners in terms of the cost of equipment, power, maintenance etc. [201, 203].

2.5.2 Process of recuperative anaerobic digestion

As demonstrated as Figure 2.5, recuperative thickening provides an additional process to anaerobic digestion, which returns thickened sludge to the digester. During the recuperative anaerobic digestion, solids from the digestate were separated by thickening equipment, and reintroduced back into the digester with incoming solids [67]. This process separates HRT and SRT in the anaerobic digestion, providing longer SRT than HRT. Recuperative process can enhance the performance of anaerobic digestion by returning active bacteria back to the digester and elutriate inhibitory metabolic by-products during the thickening process [67]. The thickening technologies such as gravity thickening, centrifugal and anoxic gas flotation technologies have been installed at full-scale recuperative anaerobic process [189]. Recuperative anaerobic process was first introduced in 1967 by Torpey and Melbingerin at Bowery Bay Plant, New York city, USA [35] and the authors observed increase volatile matter destruction and biogas production [35]. Sludge flow recycling ratio (recycle flow rate/influent flow rate) (R) and sludge concentration recycling ratio (recycle sludge TVS concentration/reactor TVS concentration) (C) were reported to be two of the operation parameters that influence recuperative anaerobic digestion. Yang et al. [206] utilized swine wastewater sludge as substrate and reported the optimum ranges of R and C are

0.25-0.30 and 2.0-3.0, respectively. Meanwhile, Ouyang and Lin [207] studied controlled recirculation of anaerobic activated sludge digester, and found the most effective R and C values are 0.5 and 1.66, respectively, in the anaerobic digestion of primary/secondary sludge mixture (45/55). In a another study by Ouyang and Chang [208], anaerobic digester with sludge flow recycling ratio ranging from 1 – 3 was more stable and producing more gas than conventional or other recycling ratio digesters. In Australia, Bharambe et al. [68] studied the full-scale plant operation with recuperative thickening in Sydney. The result was showing that recuperative thickening increased SRT from 15 days to 40 days, increased biogas production by 20%, and decreased biosolids wet mass by approximately 22%. Also, recuperative thickening helped to eliminate the odour issue of biosolids.

2.5.3 Advantages and disadvantages of recuperative thickening on anaerobic digestion

As mentioned before, recuperative thickening process aims to decouple HRT from SRT, which results in increased SRT during the anaerobic digestion. The advantages of recuperative thickening include [67, 209]:

1) Anaerobic digester volume can be decreased, which may result in lower life cycle cost.
2) Extended solids retention allows for further organic conversion to methane gas because of larger number of anaerobic bacteria being returned.
3) The increased solids concentration can serve as a buffer to shock loadings.
4) Volatile solids destruction is greater than conventional mesophilic digestion.
5) Recuperative thickening increases the solids concentration to dewatering, which has been shown to increase sludge dewater ability.

On the other hand, the disadvantages of recuperative thickening are presented as follow:

1) Increased solid concentration in the digester will require greater mixing power requirement.

2) The pre-digestion thickening requires additional mechanically intensive process to hand sludge, and additional capital and operating cost is required.
3) Centrifuges and gravity belt thickeners may become a source of odours.
4) More research is needed to understand the kinetics of recycling viable microorganisms in anaerobic digesters.

2.6 Impacts from recuperative thickening

2.6.1 Oxygen exposure

The thickening techniques such as gravity belt, dissolved air flotation (DAF), anoxic gas flotation, centrifuge and gravity thickening could introduce oxygen into the anaerobic digester system, which is a concern on the viability and activity of the methanogenic microorganism. Reynolds et al. [37] established batch tests to study whether oxygen exposure had adverse effects on the methanogens in digester. Digested sludge was aerated for 15 minutes, and the biogas production was compared with untreated samples. The results showed that gas production was declined by approximately 10% by the aeration. The authors concluded that oxygen exposure had no appreciable effect on the methanogens inactivity. Conklin et al. [40] studied the effect of oxygen exposure during thickening process on the digester performance. Two different experiments evaluated the effects of different oxygen exposure levels similar to thickening techniques gravity belt and DAF on the digester performance and on acetoclastic methanogenic activity. It was found that one-time oxygen exposure from gravity belt had no apparent effect of the oxygen on the methane production. However, decreased digester acetate used capacity (by 15%) and methane production was observed when 7% of the sludge was exposed to oxygen for 4 hours a day, 5 days per week. The authors stated that minimal oxygen during gravity belt thickening did not affect the anaerobic digestion, while high concentration of oxygen exposure during thickening process like DAF, would reduce the aectoclastic capacity and lead to failure [40]. The reason why methanogenic bacteria can be tolerate to low range oxygen

exposure is that methanogenic bacteria are well protected in sludge granules, and oxygen-consuming facultative bacteria in the immobilised consortia can metabolise part of the available substrate and consume oxygen, creating anaerobic microenvironments [210].

2.6.2 Cell lysing through shearing

Cell lysis is also referred to as sludge disintegration process during which the cell walls are ruptured, leading enhanced microbial decomposition and biogas recovery. The sludge disintegration process of either the primary digester feed or the secondary digester effluent could enhance digestion of anaerobes produced by the recuperative thickening process. Cell lysis can be achieved by several types of processes such as mechanical, chemical, thermal, ultrasonic, etc. [211]. The effect of thermal hydrolysis as a pre-treatment of sludge on the anaerobic digestion has been discussed in Section 2.3.2. In this section, the cell lysis caused by physical force occurs during the centrifuge will be considered.

There have been a few studies focusing on the method of cell disintegration by means of lysis-thickening centrifuge. For example, Dohanyos et al. [41] studied a special impact gear incorporated in the thickening centrifuge in a lab-scale batch test, and the result showed that centrifuge thickened sludge gave significant increment of methane production (84.6%) compared to untreated sludge. The reason was explained that the thickening centrifuge could break sludge floc structure and wall and release cell lysate, which could accelerate degradation reactions and methane fermentation [41]. The lysate-thickening centrifuges have been applied to full-scale WWTP in Prague, Czech Republic. The operational experience showed that, the treatment increased the specific biogas production of excess sludge by 11-31% [42]. Furthermore, the implementation of lysis-centrifuge in Furstenfeldbruck and Aachen-Soers, Germany also helped to increase the biogas production by 15-26% [43].

However, other study observed negligible or negative impact of centrifuge thickening process on the methanogenic sludge viability and activity. Batstone et al. [44] studies two plants with recuperative thickening and three without, and the high speed centrifuges are used for final dewatering for all plants. The specific methanogenic activity test of dewatering feed, cake and centrate revealed that, recuperative thickening did not affect the specific methanogenic activity remarkably with only 0% - 20% decrease; while centrifuge based dewatering had a significant and variable impact on viability of methanogens with 20% - 90% decreases. In another study conducted by Batstone et al. [45], it was found that dewatering through centrifuges caused a significant loss in specific methanogenic activity (average decrease of 54%) in full-scale plant. The authors believed that thickening by centrifuge can reduce activity by lysing cells through shear, particularly at high solid [44, 45]. Some authors also reported the loss of the viability of bacterial population when shear force was applied. Deveci [46] suggested that shear forces (in a speed range of 2.01–3.35 m/s) would cause the loss in the viability of bacterial population when solid content was above 10%. Similar result was also found in the full-scale plants, high speed centrifuges led to 20% - 90% decrease of viability of methanogens, while rotary drum based recuperative thickening affected the specific methanogenic activity negligibly [44].

As discussed in Section 2.3.1, the stability and efficiency of anaerobic digestion rely on the syntrophic relationship among microbial population including hydrolysing and fermenting bacteria, specialized acidogenic and acetogenic syntrophs, and methanogenic archaea [14-19]. Environmental factors (*e.g.* operating conditions and process configurations) could lead to the variability in structure and function of microbial population, hence the performance of anaerobic digester system. Therefore, shearing was also reported to affect the stability and robustness of the microbial community function. In a study by Kundu et al. [48], archaea and bacteria were observed with significant reduction in the abundance and diversity under high hydrodynamic shear. Microbial community and granules were also affected by the

shear in the continuously stirred anaerobic digester [47, 49]. Hoffmann et al. [49] found increasing mixing influenced the competition between the acetoclastic methanogens, *M. concilii* and *Methanosarcina spp*. *Methanosarcina spp*. became more important in the intensely mixed digesters that could result in more stabilized digesters. Jiang et al. [47] also applied continuous hydrodynamic shear to a continuous stirred tank reactor (CSTR). It is found that the density of granules after sheared digester remained unchanged, and the mechanical resistance of deformed sludge granules was slightly enhanced [47].

2.7 Recuperative thickening at full-scale WWTPs

2.7.1 Gloversville and Johnstown Joint Wastewater Treatment Facility

Gloversville and Johnstown Joint Wastewater Treatment Facility (GJJWTF) was completed in 1972 with capacity of 52.3 ML/d. The influent comes from different sources, including primary leather-related manufacturers, and residential customers in Gloversville and Johnstown. The plant upgrades in early 1990s included installation of a two-sludge anaerobic digestion system, with a 5690 m^3 primary digester and 4930 m^3 secondary digester [36]. In 2001, GJJWTF launched the combined heat and power system project and renewable generation project, which used biogas as renewable resource for cogeneration and digesters heating. By the 2003, the daily biogas generation is 2.35 ML, and annual electricity production is 0.82 million kWh, which satisfies 9-12 % of the facility's requirements [36].

Prior to 2003, the primary anaerobic digester has an average SRT of 34 days [212]. In 2003, the facility began to accept cheese whey (75.6 – 113.4 KL/week) from a local cheese manufacturer, which increased to approximately 363 ML/week in 2006 [36]. The increasing high strength dairy whey loading continued to decrease the average SRT from 25 days in 2006 to 13.4 days in 2009 [212]. Therefore, the facility began the construction and operation of the recuperative thickening system from the end of 2009 in order to increase the SRT without increase the size of the digester. The recuperative

thickening system was commissioned at the facility by the summer of 2010 with a recuperative ratio of 20%. The following Table 2.12 compares the operation data of primary digester with (in 2011) and without (in 2009) recuperative thickening. There were a few parameters showing positive data after the implementation of recuperative thickening, including SRT, biogas quantity and quality and organic loading rate. By 2011, GJJWWTF is producing 95% of its own electrical demand by the combined heat and power process fuelled by anaerobic biogas. It is believed that co-digestion of dairy whey from local industries with municipal sludge and the utilization of a recuperative thickening loop was determined to be the most cost effective option and most operable system with respect to current unit operations [212].

Table 2.12 Comparison of primary digester operation data [212].

Parameter	2009 annual average	2011 annual average
Primary digester feed total solid (%)	4.3	5.5
Organic loading rate (VS kg/m^3)	2.69	3.58
SRT (days)	13.4	21.4
HRT (days)	13.4	12.6
Volatile solids reduction (%)	68.2	65.9
Digester biogas flow	5,520	11,540
Biogas production (m^3/kg VS_d)	0.55	1.15
Biogas CO_2 content (%)	43.7	42.2
Average monthly electrical generation (kWh)	142,800	408,500

2.7.2 Spokane Advanced Wastewater Treatment Plant

Spokane Advanced Wastewater Treatment Plant (SAWTP) (now City of Spokane Riverside Park Water Reclamation Facility) in Washington, USA is a result of advancements in wastewater treatment technology since 1958. Over the years, a series of improvements have been made to upgrade the treatment procedures and capacity.

The latest upgrade project was finished in December 2011, leading the treatment capacity increased to 91.2 ML/d. The new plant enhances the county's wastewater treatment capability and serves the projected population growth. It also improves the water quality in the region and reduces the phosphorus discharges to the Spokane River. CH2M HILL investigated the potential benefits of recuperative thickening on the two full-scale anaerobic digesters (diameter of 30.5 m) during September 11, 2000 and June 2001. In additional, one set of dissolved air flotation thickening (DAFT) co-thickening was operated to provide thickened digested solid and waste activated sludge for digester No. 2 [37].

During the test period, recuperative thickening process was token place from September to October 2000, and from November 2000 to April 2001, respectively. The operation data between August and September 2000, and between November 1999 and April 2000 were used as comparison. The following Table 2.13 lists plant performance with and without recuperative thickening during test period.

Table 2.13 Performance comparison of anaerobic digestion with and without recuperative thickening [37].

Parameters	Test 1 Period		Test 2 Period	
	1 Aug - 11 Sep, 2000 Without recuperative thickening	11 Sep - 17 Oct, 2000 With recuperative thickening	Nov 1999 - Apr 2000 Without recuperative thickening	Nov 2000 – Apr 2001 With recuperative thickening
Digester No. 2 TS (%)	2.48	2.38	2.53	2.86
Digester No. 2 VS (%)	1.29	1.18	1.62	1.80
Digester No. 2 HRT (d)	9.4	8.1	8.1	10.5
Digester No. 2 SRT (d)	9.4	9.5	8.1	13.2
Digester No. 2 pH	7.08	7.04	7.10	7.09
Digester No. 2 alkalinity (mg/L)	3,345	3,451	3,847	3,577
Digester No. 2 volatile acid (mg/L)	476	560	439	429
Digester No. 1 TS (%)	2.29	2.19	2.31	2.71
Digester No. 1 VS (%)	1.14	1.02	1.42	1.65
Digester No. 1 HRT (d)	9.3	7.7	7.5	8.7
Digester No. 1 SRT (d)	9.3	9.2	7.5	10.9

Parameters	Test 1 Period		Test 2 Period	
	1 Aug - 11 Sep, 2000 Without recuperative thickening	11 Sep - 17 Oct, 2000 With recuperative thickening	Nov 1999 - Apr 2000 Without recuperative thickening	Nov 2000 – Apr 2001 With recuperative thickening
Digester No. 1 pH	7.21	7.18	7.22	7.17
Digester No. 1 alkalinity (mg/L)	3,602	3,781	4,395	3,933
Digester No. 1 volatile acid (mg/L)	563	572	397	502
Anaerobic digestion total HRT (d)	18.8	15.8	15.7	19.2
Anaerobic digestion total SRT (d)	18.8	15.8	15.7	24.0
Anaerobic digestion VS removal (%)	61.9	69.3	50.3	64.4
Average polymer consumption (kg/d)	168	163	157	133
Secondary effluent BOD (mg/L)	5.3	6.5	12.0	10.4
Secondary effluent TSS (mg/L)	5.8	7.2	9.4	8.1

The first test only last 37 days, and approximately 20 -25% of the digested solids were recuperative thickened with waste activated sludge in the DAFT. The second test was operated with the same condition of the first one, and the results were compared to the operating data during a similar period when recuperative thickening was not being tested. However, the short period of the first test may not be long enough to allow the digesters to be stable; the major advantages of recuperative thickening were found in the second test:

1) Recuperative thickening decoupled SRT from HRT, and increased total SRT of anaerobic digestion by about 50%.
2) Recuperative thickening had no effect on effluent quality.
3) Operating parameters, such as pH, alkalinity and volatile acid were not significantly different.
4) Anaerobic digester TS was increase by recuperative thickening
5) Anaerobic digestion VS removals were increased by 10% to 14% by using recuperative thickening in the two tests.
6) Although co-thickening requires more polymer consumption for DAFT; total polymer consumption (the sum of thickening and dewatering) was less when recuperative thickening was implemented.

The operation of recuperative thickening in SAWTP shows that co-thickening with waste activated sludge can be accomplished without an increase in labour and power requirements. It also demonstrates that the use of recuperative thickening does not affect the effluent quality, digester pH, alkalinity etc., but the digester SRT and VS removal are remarkably increased.

2.7.3 Bondi wastewater treatment plant

Bondi WWTP is one of the three largest WWTPs in Sydney, NSW, Australia, treating an average of 120 million litres per day and serving a population of approximately half a million people. Bondi WWTP is a high-rate primary treatment plant with four digesters. Two digesters were operated in parallel as primary digesters, followed by two phases of secondary digesters (Figure 2.6). Recuperative thickening was applied between secondary digester, and thickened sludge was recirculated to the primary digester with inlet of primary sludge. Since 2008, recuperative thickening started to be implemented in Bondi WWTP, and a research conducted by Bharambe et al. [68] reviewed 3 years of operation data and evaluated the impact of recuperative thickening on plant operation.

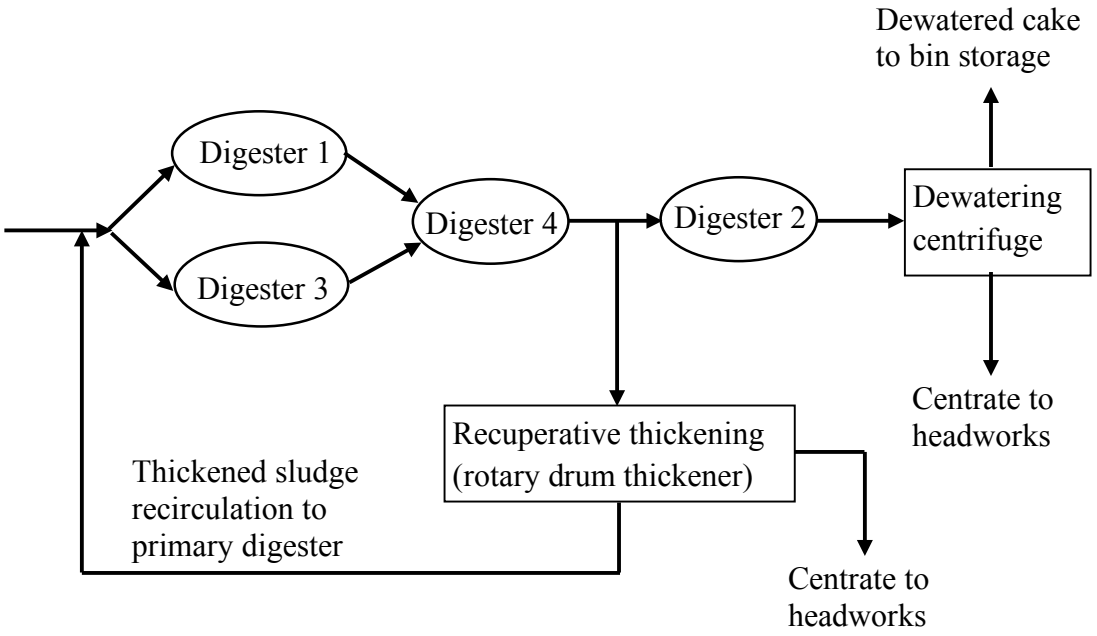


Figure 2.7 Bondi recuperative thickening simplified process (obtained from Bharambe et al. [68]).

Operational data obtained from July 2007 to July 2010 showed that average SRT of digestion system was increased from approximately 15 days to 40 – 50 days by implementation of recuperative thickening, and the VS removal was increased from approximately 60% to 85% accordingly [68]. Bharambe et al. [68] also found that recuperative thickening was effective to reduce the biosolid produced by approximately 22% as well as odorous compounds in dewatered cake. By comparing the concentration of H_2S, dimethyl sulphide, mercaptans in biosolid after 48 hours storage, it was found that H_2S was almost removed (>99%), and dimethyl sulphide and mercaptans were more than 80% removed by recuperative thickening [68].

2.8 Summary

Wastewater sludge, which is generated during wastewater treatment processes, need to be treated and stabilised before disposal. Biological processes have been widely used in full-scale WWTPs for sludge treatment; and among them, anaerobic digestion is considered as the most effective and economic approach. This chapter reviews the biochemical mechanism of anaerobic digestion, factors influent digester performance, risks associated with biosolids disposal and recuperative thickening for a modified digestion process.

Anaerobic digestion is a microbial mediated process, which can convert organic matters to biogas via four major steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis.

Anaerobic digestion can achieve up to 40% of TS removal of feed sludge and produce usable energy source (methane) as a major product. As been reviewed in this chapter, there are several factors can affect the performance of anaerobic digesters, including digester temperature, pre-treatment methods, pH value, alkalinity, and nutrients etc.

Land application is a beneficial approach for treated sewage sludge (biosolid) disposal. However, pollutants which are resistant to anaerobic digestion could lead problem of community health and environmental justice. Major concern of sludge disposal comes from sludge odour, risks of heavy metal, and residue of TrOCs. Previous studies have showed several methods to address such risks. One of the methods is recuperative thickening, which thickens and returns part of the digestate to the digester to extend SRT from HRT, providing possibility to increase biogas production and improve biosolids quality. In addition, recuperative thickening could increase the treatment digestion capacity without the need for additional space and excessive capital expenditure, which provides the solution for increasing treatment capacity demand due to urbanization and population growth. However, due to the additional thickening process, there are a few inevitable impacts for digester performance, such as oxygen exposure to the methanogens and sludge shearing caused by thickeners. Previous studied have revealed that low level of oxygen exposure caused by thickening had no appreciable effect on the methanogens inactivity, and sludge shearing could impose significant effect on the microbial community structure thus the digestion performance. Recuperative thickening has been applied in some full-scale WWTPs in USA and Australia. Results shows that SRT of digesters, biogas production, plant net electricity production, biosolids reduction have been improved due to recuperative thickening.

Chapter 3 Methodology

Lab-scale anaerobic digesters were used in this project. This chapter will describe the experiment apparatus, materials, experimental plans and analytical methods involved in the whole project.

3.1 Sludge used for the project

For all experiments conducted in this project, primary sludge and anaerobically digested sludge were taken from Wollongong Wastewater Treatment Plant (WWTP), NSW, Australia. Primary sludge was taken from the primary sedimentation tank of the plant fortnightly and stored in fridge under 4 ˚C until use. It is notable that the total solid (TS) content of primary sludge was varied between 20 g/L and 30 g/L, which was subjected to the influent of plant and weather conditions. Anaerobically digested sludge was collected from the full-scale anaerobic digestion of the same plant. Primary sludge was used as feed for the lab-scale digesters, and anaerobically digested sludge would be seeded to the digester as inoculum immediately after sampling.

3.2 Lab-scale anaerobic digesters

Three identical lab-scale anaerobic digesters were used for this project. Each digester consisted of a 28 L stainless steel reactor (Core Brewing Concepts, Victoria, Australia), a peristaltic hose pump (DULCO® flex from ProMinent Fluid Controls, Australia), a temperature control unit (Neslab RTE 7), a thermal couple with temperature gauge, a biogas counter and a gas trap for biogas sampling (Figure 3.1a). The digester was heated by hot water from the temperature control unit flowing inside plastic tubes which were wrapped around the digester. The entire reactor was also wrapped with insulation foam to keep stable temperature (35 °C) of digester. The thermal couple probe was kept in the sludge to test the reactor temperature. The gas line was connected to gas trap though the gas meter which could be used to record the gas production of digester over a certain period. The gas trap is partially filled with water, and connected to an open water tank. Biogas could be trapped in when the gas out valve is closed, and used for biogas composition analysis. Otherwise, the biogas would be emitted to an open area outside the lab through tubing. It is noteworthy that there was an air-lock connected to the gas out valve in order the prevent oxygen entering the digester. The Figure 3.1 shows the schematic diagram of individual digester (Figure 3.1 a) and the photo of three digesters working parallel in the lab (Figure 3.1 b).

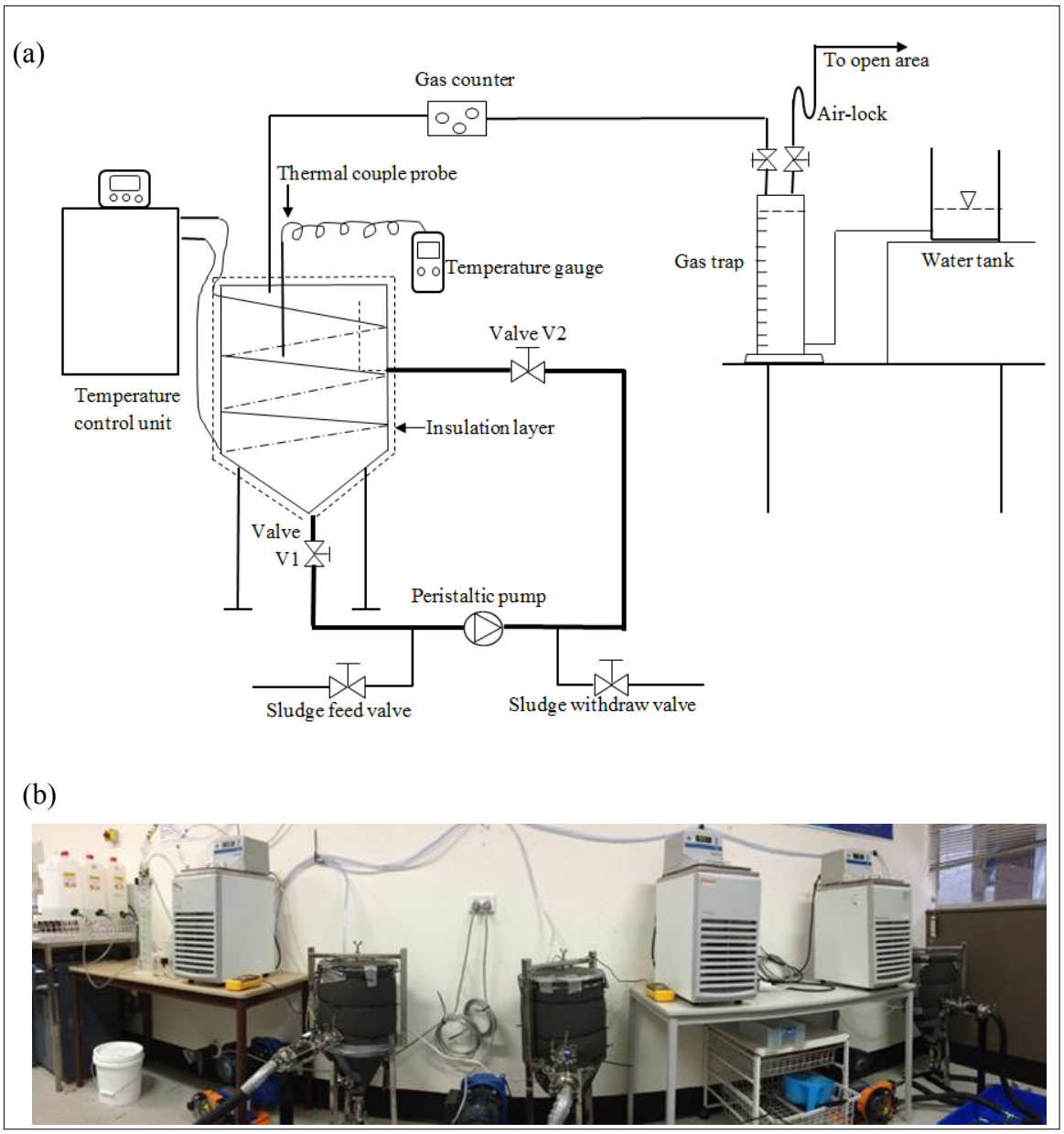


Figure 3.1 (a) Schematic diagram of individual digester and (b) photo of the three digesters.

All three anaerobic digesters were seeded with 20 L digested sludge individually at the beginning of each experiment. The peristaltic hose pumps were operated at a flow rate of 60 L/h, which played the role of sufficient mixing for the digesters. The peristaltic pumps were also used to withdraw waste sludge and feed sludge daily. According to different experimental plans, predetermined amount of digested sludge was withdrawn, wasted/return to digester after treatment; and primary sludge was fed into the digesters. Detailed feeding regime will be presented in the following sections.

3.3 Experimental schemes

The project consisted of four studies regarding the anaerobic digester performance in biogas production, solids removal, TrOC removal and microbial community structure under different conditions. The following flow chart provides the overview of all studies.

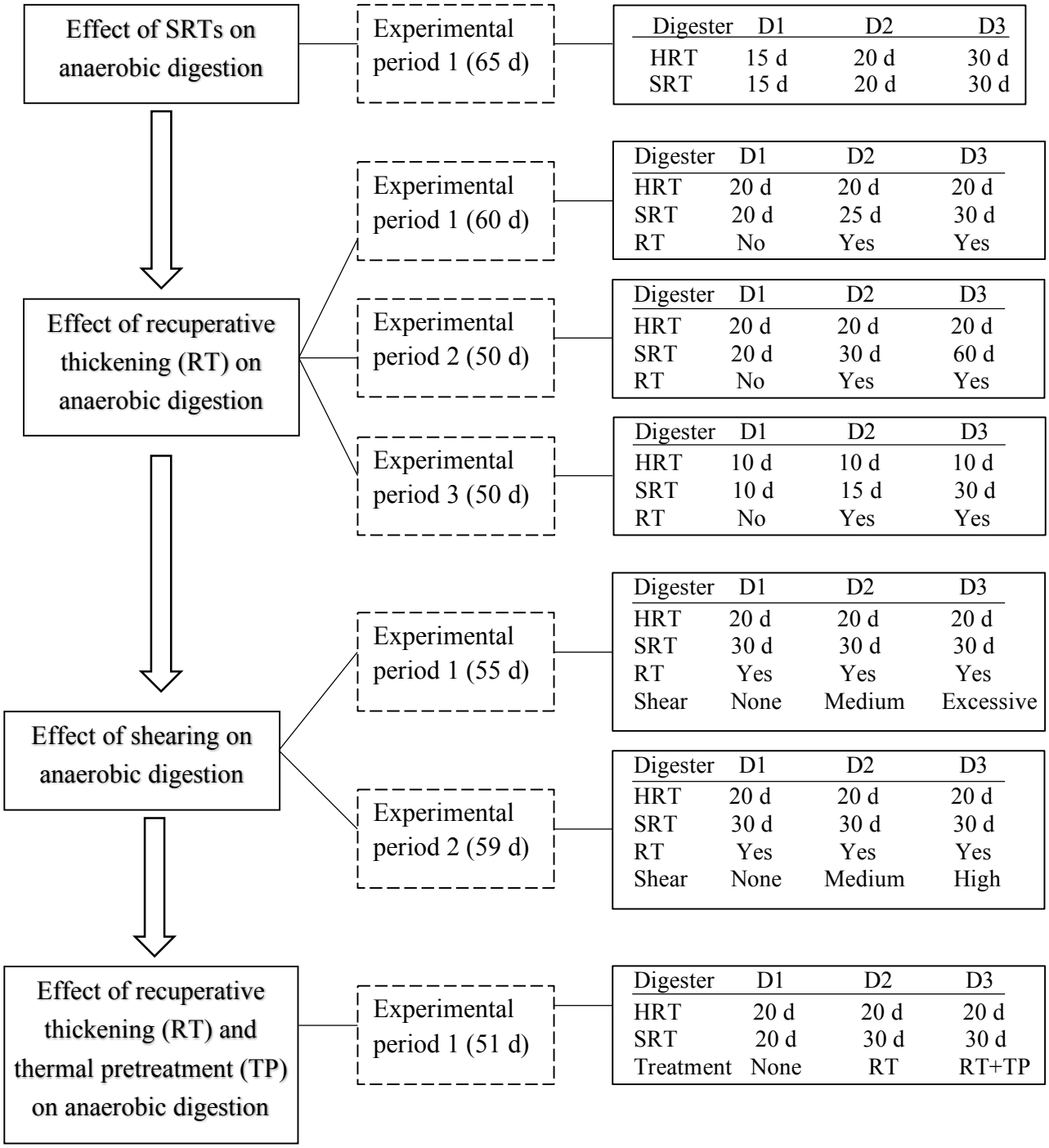


Figure 3.2 Flow chart of all studies in this project

The following sections 3.3.1 – 3.3.4 will describe the detailed experimental regimes, monitored parameters and sampling frequency, etc.

3.3.1 The study for the effect of sludge retention time (SRT) on the anaerobic digestion performance and trace organic contaminants (TrOCs) removal

Three digesters were seeded with 20 L of anaerobically digested sludge taken from Wollongong WWTP. Digesters namely D1, D2 and D3 were operated under SRT of 15 days, 20 days and 30 days, respectively. Certain amount of the digested sludge was wasted first, and then same volume of primary sludge was fed into digester daily. As a result, the HRT and SRT were same. Table 3.1 shows the detailed feeding regime for this experiment.

Table 3.1 Feeding regime for anaerobic digestion with different SRTs.

Digester	D1	D2	D3
HRT (d)	15	20	30
SRT (d)	15	20	30
Wasted sludge (L/d)	1.33	1	0.67
Primary sludge (L/d)	1.33	1	0.67

Throughout the experiment, the biogas production of each digester was monitored everyday by the online gas counter, and biogas samples were collected for the analysis of biogas composition weekly. Sludge samples of primary sludge and digested sludge from each digester were taken weekly in order to analyse the sludge parameters, including TS, volatile solid (VS), total chemical oxygen demand (tCOD), soluble chemical oxygen demand (sCOD), alkalinity and pH. Additional sludge samples were taken every 10 days in order to prepare the TrOC concentration analysis. Analytical method will be discussed in Section 3.4.

3.3.2 Recuperative thickening

This study consisted of three experiments; during which recuperative thickening was applied to achieve different SRTs without increasing the HRT. Throughout all three experiments, digester D1 was operated as a control system without recuperative thickening to achieve same

SRT and HRT all the time, and digester D2 and D3 were operated with recuperative thickening to achieve extended SRTs. The detailed feeding regime is indicated in Table 3.2.

Table 3.2 Feeding regime for anaerobic digesters with recuperative thickening.

	Experiment 1			Experiment 2			Experiment 3		
	D1	D2	D3	D1	D2	D3	D1	D2	D3
Recuperative thickening	No	Yes	Yes	No	Yes	Yes	No	Yes	Yes
Active volume (L)		20			20			20	
HRT (d)		20			20			10	
SRT (d)	20	25	30	20	30	60	10	15	20
Withdrew sludge (L/d)	1	2	2	1	2	2	2	3	3
Wasted sludge (L/d)	1	0.8	0.67	1	0.67	0.33	2	1.3	1
Thicken ratio	None	1.2	1.33	None	1.33	1.67	None	1.67	2
Primary sludge (L/d)		1			1			2	

Throughout the experiment 1, digest D1 was operated as a control system with both of SRT and HRT of 20 d, and digester D2 and D3 were operated with recuperative thickening to achieve SRT of 25 d and 30 d, respectively. Certain amount of anaerobic digested sludge was withdrawn from the digesters each day (Table 3.2). For digester D2 and D3, part of the sludge was discharged as waste sludge (Table 3.2), and the rest was conditioned with dewatering polymer (Zetag® 8165, BASF) at concentration of 7.5 g/kg dry sludge. Treated sludge was settled for 5 minutes and supernatant was separated in order to achieve determined thicken ration (Table 3.2), and then 1 L of thicken sludge was returned to the digester with feed sludge (primary sludge). As a result, D2 and D3 could have SRT of 25 d and 30 d, respectively while their HRTs remained at 20 days. The operation of experiment 2 was similar to experiment 1, except the wasted sludge amount and thicken ratio of digester D2 and D3 were changed according to Table 3.2. The SRTs of D2 and D3 reached 30 days and 60 days, respectively, with the same HRT (20 days) of control digester D1. The experiment 3 studied lower range of SRTs for anaerobic digestion. Control digester D1 was operated at both SRT and HRT of 10 days. D2 and D3 achieved SRT of 15 d and 20 d, respectively by recuperative thickening. The volume of wasted sludge and thicken ratio was shown in Table 3.2.

The digester performance parameters were monitor for all experiments, including biogas production, biogas composition, TS, VS, tCOD, sCOD, alkalinity, pH etc. At the end of each experiment, digested sludge was taken to prepare dewatered sludge for odour analysis. The analytical method will be discussed in Section 3.4 in details.

3.3.3 Sludge shearing

This experiment was designed to study the impact of shearing occurred during thickening process on the anaerobic digesters performance, the microbial community structure and the TrOCs fate during digestion.

All three digesters were operated with recuperative thickening to achieve an SRT of 30 days while maintaining a HRT of 20 day. Table 3.3 elucidates the operational regime for the experiment. Each day, 2 L of digested sludge was extracted from the digester, and 0.67 L was wasted. Thickening polymer (Zetag 8169, BASF) was added to the remaining digested sludge at a dose of 7.5 g/Kg dry sludge for thickening. Then supernatant was wasted to obtain 1 L of thicken sludge, which would be sheared at different levels according to the experimental plan. Digester D1 was the control system with gentle stirring (designated as no shearing) during the thickening process. At the same time, shearing was applied to thickened sludge from digesters D2 and D3 (Table 3.3). A mixer (Servodyne mixer head, model 50003-25, Boronia, Australia) with a 2-blade bending paddle impellor (5 cm x 10 cm) was used to provide medium (300 rpm, comparable to a rotary drum) and high (600 rpm, significantly higher than the shearing induced by a rotary drum) shearing to the thickened sludge from D2 and D3, respectively. A food blender (Sunbeam, model PB9500, Australia) was also used to simulate excessive shearing to the thickened sludge from digester D3 (Table 3.3). In all cases, the shearing process lasted 5 minutes.

Digester D3 was regenerated at the end of second experimental period by renewing part of its sludge. 5 L of the digested sludge from D3 was replaced by freshly collected anaerobically digested sludge from wastewater treatment plant (Period 3), and another 5 L of the digested sludge was replaced again in 2 weeks' time (Period 4). During these 2 periods, no shearing was applied to thickened sludge from D3.

Table 3.3 Operational regime for anaerobic digestion with/without shearing.

Operational parameters	Period 1 (Day 1 – 55)			Period 2 (Day 56 – 114)			Period 3 & 4 (Day 115 – 142)		
	D1	D2	D3	D1	D2	D3	D1	D2	D3
Shearing	None	300 rpm	Blender	None	300 rpm	600 rpm	None	300 rpm	None
Recuperative	Yes			Yes			Yes		
HRT (d)	20			20			20		
SRT (d)	30			30			30		
Withdrew sludge (L/d)	2			2			2		
Wasted sludge (L/d)	0.67								
Thicken ratio	1.33								
Primary sludge (L/d)	1								

Anaerobic digester performance parameters mentioned in section 3.3.1 and 3.3.2 were also monitored during this experiment. Additionally, samples from digested sludge and primary sludge were taken weekly to prepare TrOCs samples. At the end of period 1 (day 55) and period 2 (day 110), digested sample from each digester were taken for DNA extraction and 16S rRNA gene amplicon sequencing in order to analyse the microbial community structure.

3.3.4 Thermal pre-treatment

Thermal pre-treatment of feed sludge has been approved to be an effective way to improve biogas production and digester performance. Thus, another experiment was carried out to study how thermal pre-treatment affected the anaerobic digestion performance and the removal of TrOCs.

Digester D1 was the control system with same SRT and HRT (20 days). Each day, 1 L of the digested sludge from D1 was wasted and 1 L of original primary sludge was fed in order to maintain the determined SRT and HRT. The primary sludge fed to digester D2 and D3 was thermally pretreated prior to feeding. Digester D2 was operated with same feeding regime with D1, while digester D3 was operated with recuperative thickening to achieve SRT of 30 d and

HRT of 20 d. The thickening process was the same described in section 3.3.23. Table 3.4 listed the digesters operational regime of this study.

Table 3.4 Operational regime for anaerobic digestion with thermal pre-treatment.

Digester	D1	D2	D3
SRT (d)	20	20	30
HRT (d)	20	20	20
Recuperative thickening	No	No	Yes
Withdrawn sludge (L/d)	1	1	2
Wasted sludge (L/d)	1	1	0.67
Thicken ratio	None	None	1.33
Feed sludge (L/d)	1	1	1
Thermal pre-treatment	No	150 °C, 30 min	150 °C, 30 min

Primary sludge fed for digester D2 and D3 was treated before feed procedure every day. The primary sludge was thermally treated by using a 5 L pressure vessel with heating jacket and mixer (Figure 3.3). Original primary sludge was sealed in the vessel, and the vessel was heated to 150 °C with internal pressure of 500 kPa, then heating process lasted for 30 min. After the heating process, the pressure inside the vessel was released gradually through the pressure release valve, and the sludge was cooled to room temperature. Then, 1 L of the treated primary sludge was fed to D2 and D3, respectively.

The biogas production and composition was monitored through the experiment. Sludge samples from raw primary sludge, thermal treated primary sludge and digested sludge were take weekly to analysis the sludge characteristic parameters including TS, VS, tCOD, sCOD, pH, alkalinity etc. Additional sludge sample were taken from primary sludge and digested sludge to prepare for TrOC concentration analysis.

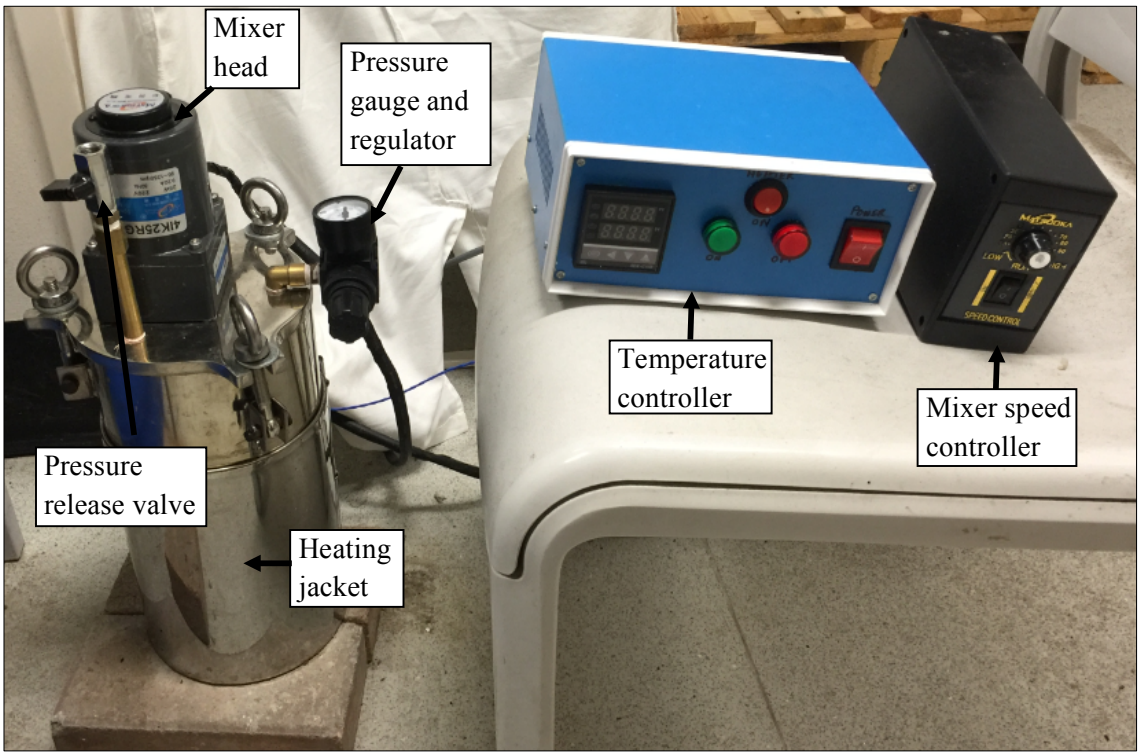


Figure 3.3 Pressure vessel used for sludge thermal treatment.

3.4 Analytical methods

3.4.1 Biogas production and composition

The quantity and quality of biogas are important parameters to evaluate the sufficiency of anaerobic digestion. As mentioned before, biogas production of each anaerobic digester was recorded separately through individual gas counter during a certain period (approximately 24 hours), and average gas flow is calculated for each system every day. It is needed to note the biogas flow could vary between every feed cycle (every 24 hours). For example, the higher production could be observed during a short time after feeding, and the production could decrease after a few hours. Therefore, the average biogas flow during each day is more stable and reliable for evaluation of the biogas production. The gas trap was used for collecting biogas when the gas in valve was open and gas out valve was closed (Figure 3.1a). When enough biogas was stored (approximately 700 mL), a portable gas analyser (GA5000 gas analyser, Geotechnical Instruments (UK) Ltd, England) was connected to the air lock with the gas out valve open in order to measure the composition of biogas. The gas analyser measures the composition of CH_4, CO_2, and O_2 in percentage and H_2S in ppm.

3.4.2 Sludge character parameters

Sludge samples are taken weekly from each digester as well as the primary sludge. The tested sludge characters include TS, VS, tCOD, sCOD, pH, alkalinity (exclude primary sludge). All samples are stored in the fridge at 4 ˚C, and measured within one week after sampling.

The pH of sludge sample is measured by the pH meter (Orion 4 Star pH and conductivity portable meter, Thermo Scientific, Australia). The values of TS, VS, alkalinity are measured in accordance to the standard methods for the examination of water and wastewater, 21st edition [213]. COD is measured by the high range plus COD reagent (HACH Company, USA) following the reactor digestion method 8000 from Hach Water Analysis Handbook. The removal of TS, VS, tCOD or sCOD is calculated by the equation:

$$Removal = \left(1 - \frac{value\ of\ digested\ sludge}{value\ of\ raw\ sludge}\right) * 100\% \quad \text{(Equation 3-1)}$$

The supernatant used for measurement of sCOD is obtained by centrifuging at 3720xg for 10 minutes (Allegra X-12R centrifuge, Beckman Coulter, Australia), and then filtration by 1 μm glass microfiber filter paper (Filtech, Australia).

Beside the removal of COD, the tCOD mass balance was introduced to demonstrate the COD consumption pathways for each digestion during the experiment. Theoretically, the amount of tCOD converted to methane gas can be calculated as

$$COD_{methane} = 2 * m_{methane} * M_{oxygen} \quad \text{(Equation 3-2)}$$

where $m_{methane}$ is the molar volume of methane in the biogas (moles), M_{oxygen} is the molar density of oxygen (g/mol). The ideal gas law is used to determine the molar volume of methane. The COD of influent can be denoted as

$$COD_{influent} = tCOD_{primary\ sludge} * V_{primary\ sludge} \quad \text{(Equation 3-3)}$$

where $V_{primary\ sludge}$ is the volume of feed sludge per day (L). And the COD of the sludge effluent can be de denoted as

$$COD_{effluent} = tCOD_{digested\ sludge} * V_{waste\ sludge} \quad \text{(Equation 3-4)}$$

where $V_{waste\ sludge}$ is the volume of wasted digested sludge per day (L). Thus the balance of COD consumption for each digester can be described as

$$COD_{influent} = COD_{methane} + COD_{effluent} + COD_{accumulation} \quad \text{(Equation 3-5)}$$

where $COD_{accumulation}$ is the COD accumulated in the digester.

3.4.3 Sludge dewatering and odour measurement

At the end of each experiment, digested sludge from each digester was collected to prepare dewatered sludge samples and sent for odour measurement, which measures the concentration of volatile organic sulphur compounds (VOSCs) in the dewatered biosolids. The tested VOSCs include hydrogen sulphide, methyl mercaptan, ethyl mercaptan, dimethyl disulphide, carbon disulphide, dimethyl sulphide, carbonyl sulphide. Digested sludge freshly taken from the digester was dosed with dewatering polymer (Zetag® 8165, BASF) at the concentration of 7.5 g/kg dry solid and mixed well. Then the treated sludge was transferred to a customer designed centrifuge tube (Figure 3.4) and centrifuged at 3720xg for 20 minutes (Allegra X-12R centrifuge, Beckman Coulter, Australia) in order to obtain dewatered biosolid with approximately 20% dry solid content. Certain amount (60 g) of the biosolid cake was stored in a sealed bottle (250 mL), and sent to Sydney Water West Ryde laboratory for VOSCs analysis. For the analysis, the biosolid cake was incubated at 25 °C within the sealed bottle, and the air from the bottle head space was sampled regularly to analysis the concentration of VOSCs.

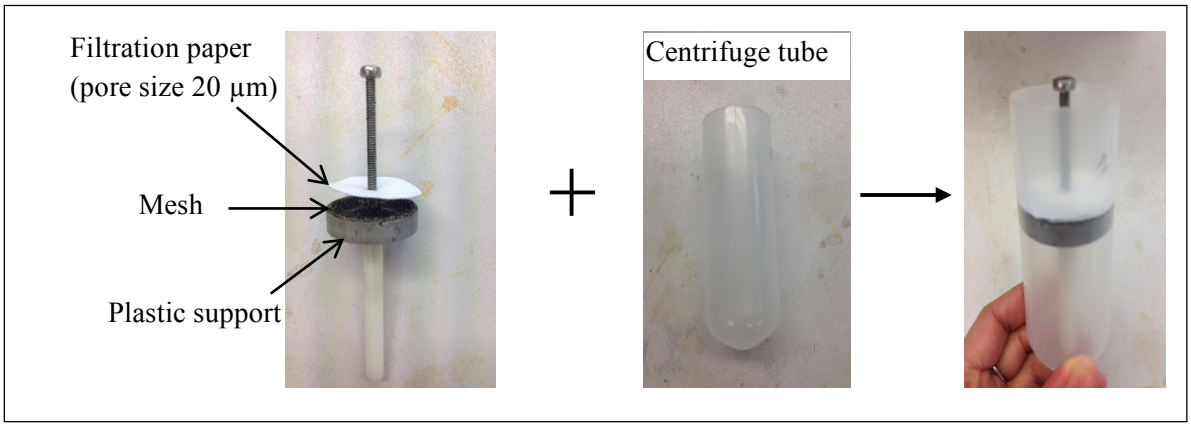


Figure 3.4 Sludge dewatering apparatus (modified from study by Higgins et al. [214]).

3.4.4 Trace organic contaminants (TrOCs) concentration analysis

Preparation of samples for TrOC analysis was followed the similar way described by a previous study [215]. Sludge samples from primary sludge and digested sludge were centrifuged at 3720xg for 10 minutes (Allegra X-12R centrifuge, Beckman Coulter, Australia) to separate solid pellets and supernatant for further analysis. Supernatant (50 mL) from the sludge sample was diluted to 500 mL by Milli-Q water and filtered by 1 µm and 0.7 µm pore size glass microfiber filter paper for solid phase extraction (SPE). The pellet from the sludge samples

were freeze dried for 10 hour using the Alpha 1-2 LDplus freeze Dryer (Christ GmbH, Germany). The dried samples were then grounded to power and 0.5 g powder was transferred to a 13 mL glass vial with cap for extraction.

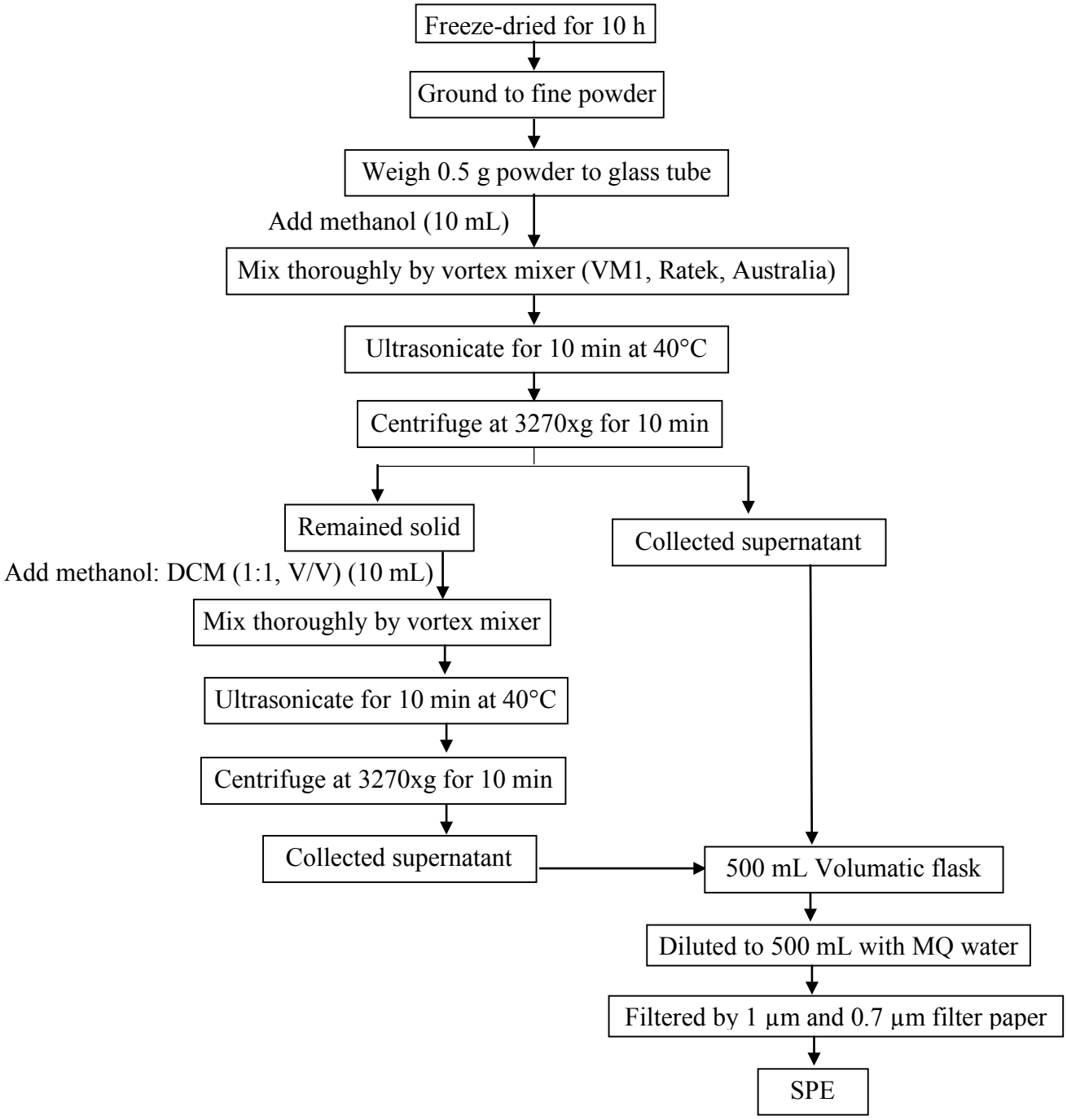


Figure 3.5 Procedures of extracting TrOCs from solid phase sludge.

Figure 3.5 demonstrates the procedures for solid sample extraction. Methanol (10 mL) and the solvent made of dichloromethane (DCM) and methanol (1:1, v/v) (10 mL) were used for the

extraction. The extracted liquid was diluted to 500 mL by Milli-Q water, and then filtered by 1 μm and 0.7 μm pore size glass microfiber filter paper for subsequent SPE.

The extracted liquid samples from both the sludge supernatant and solid were spiked with surrogate (50 μL per sample) with all analytes for method recovery and detection level determination. The 200 mg Oasis HLB cartridges (Waters, Milford, MA, USA) were conditioned with 5 mL methyl tert-butyl ether, 5 mL methanol, and 2 x 5 mL Milli-Q water before subsequence SPE. The liquid samples were loaded onto the cartridges at the flow rate of approximately 15 mL/min. Then the cartridges were dried with a stream of nitrogen for 45 minutes, and stored at -15 ^{o}C until analysis. Analytes were eluted from the cartridge with dichloromethane (4 - 3 mL) into 20 mL glass tubes. Samples were analysed on an Agilent 7890A gaschromato-graph (GC) coupled with an Agilent7000B triple quadrupole mass spectrometer (MS/MS). The details of the GS-MS-MS analytical method was described in previous study by McDonald et al. [216].

In order to understand the fate of TrOCs during the anaerobic digestion, the TrOC mass balance was used to calculate the amount of TrOC which was biodegraded, absorbed on the solid phase and residual in the aqueous phase. The following equations describe the mass calculation.

The inlet TrOC concentration can be denoted as

$$C_{in} = X_{in} \times TS_{in} + S_{in} \quad \text{(Equation 3-6)}$$

where C_{in} is the total inlet concentration (ng/L), X_{in} is the TrOC concentration in the solid phase of primary sludge (ng/g dry sludge), TS_{in} is the total solid concentration of primary sludge (g/L), and S_{in} is the TrOC concentration in the aqueous phase of primary sludge (ng/L). Similarly in the outlet sludge, the concentration of TrOC can be calculated as

$$C_{out} = X_{out} \times TS_{out} + S_{out} \quad \text{(Equation 3-7)}$$

where C_{out} is the total outlet concentration (ng/L), X_{out} is the TrOC concentration in the solid phase of digested sludge (ng/g dry sludge), TS_{out} is the total solid concentration of digested sludge (g/L) and S_{in} is the TrOC concentration in the aqueous phase of digested sludge (ng/L). Thus the mass balance for TrOC concentration can be presented as

$$C_{in} = C_{out} + C_{bio} \quad \text{(Equation 3-8)}$$

where C_{bio} is the portion of TrOC that has been biodegraded.

3.4.5 DNA extraction and Illumina sequencing

As mentioned in 3.3.3, duplicate samples of digested sludge were collected from three anaerobic digesters at the end of experimental period 1 (Day 55) and 2 (Day 110) (Table 3.2). DNA extraction was conducted immediately using the FastDNA spin kit for soil (MP Biomedical, NSW, Australia). DNA quality was assessed using 1% agarose gel electrophoresis and Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Detailed description of this DNA extraction procedure is available elsewhere [217].

Extracted genomic DNA was submitted to the Australian Genome Research Facility (Brisbane, QLD, Australia) for amplicon sequencing on the Illumina MiSeq platform, utilizing Illumina's Nextera XT Index's and Paired End sequencing chemistry. V3-V4 variable regions of microbial 16S rRNA gene were targeted using primer pairs: 341F (5'–CTAYGGGRBGCASCAG–3') and 806R (5'–GGACTACNNGGGTATCTAAT–3').

Amplicon sequences were processed using the QIIME (version 1.9.1) [218] and USEARCH (version 8.1.1861) [219] software packages. Paired-end reads were merged using fastq-join method with minimum overlap of 100 bp. Primers were trimmed using QIIME script. The Fastq file of trimmed sequences was processed following UPARSE pipeline: quality filtering (maximum error rate of 0.5; sequences were trimmed to 240 bases and any with less than 240 bases excluded), discarding full length duplicates, abundance sorting, disposing singletons and chimera filtering. Sequences were clustered into operational taxonomic units (OTUs) and reads were then mapped back to OTUs with a minimum identity of 97%. Taxonomy was assigned by *uclust* [220] using Greengenes database (version 13_8, Aug 2013) in QIIME. Representative sequences were aligned using PyNAST [221]. Aligned sequences were filtered the gaps and then used to generate phylogeny tree by method FastTree [222].

After quality filtering, removing chimeric and singletons, 1959237 paired-end reads were obtained for total samples with sequence statistics of 110024/268601/139033.5/163269.8/46707.3 (min/max/median/mean/std, respectively). A total of 3051 operational taxonomic units (OTUs) at 97% sequence similarity were assigned. For summary of microbial composition, OTU with an abundance of less than 0.05% was removed and duplicate samples were collapsed to get the mean value.

For α and β-diversity analysis, to eliminate the heterogeneity caused by having different numbers of sequences among the samples, equivalent numbers of sequences were subsampled by rarefaction (10 iterations) to the lowest number of sequences (110000 sequences) found a

mong the samples. Specifically, α-diversity comparisons were determined using observed species, phylogenetic diversity (PD_whole_tree) and Simpson index. Good's coverage was calculated to assess the completeness of sampling and the possibility that an amplicon sequence selected randomly has already been sequenced. For β-diversity comparison, a weighted UniFrac distance metric [223] was constructed and then visualized via PCoA (Principal Coordinate Analysis). All analyses were implemented in QIIME. All sequencing data in this study are available at the Sequence Read Archive with accession number (SRP074867) in the National Centre for Biotechnology Information.

Chapter 4 The impact of SRTs on anaerobic digestion performance and TrOCs occurrences and their removals

Three lab-scale digesters described in Chapter 3, Section 3.2 were operated under SRT of 15 d, 20 d and 30 d, respectively in this study. As described in section 3.3.1, digester D1, D2 and D3 were fed with 1.33 L, 1 L and 0.67 L of primary sludge each day, respectively; which resulted in that the HRT was equal to SRT for each individual digester. Basic biological performance parameters of anaerobic digesters, including biogas production, the removals of TS, VS and COD, pH and alkalinity were systematically examined by the method described in Chapter 3, Section 3.4. Additionally, TrOCs concentrations in the aqueous and solid phase from both primary and digested sludge were quantified, and their fate during anaerobic digestion will be elucidated in this Chapter.

4.1 Anaerobic digester performance

4.1.1 Biogas production and composition

Biogas production rate and composition are key parameters to examine the anaerobic digester performance. Based on the biogas production and VS removed from each digester, the methane yield during the experiment was demonstrated in Figure 4.1. As the SRT was increased from 15 to 30 d, a notable increased in methane production activity from 0.23 to 0.69 L CH_4/g $VS_{removed}$ was observed (Figure 4.1). In previous studies, mesophilic anaerobic digesters produced approximately 0.15 L of CH_4/g $VS_{removed}$ at SRT of 14 d [24], and higher methane production (0.4 - 0.5 L CH_4/g $VS_{removed}$) was observed for digesters with SRT over 25 d [98, 128, 131]. On the other hand, biogas composition was not affected by the digester SRT. Indeed, all biogas samples were composed of approximately 60% methane and 40% carbon dioxide regardless of the digester SRT.

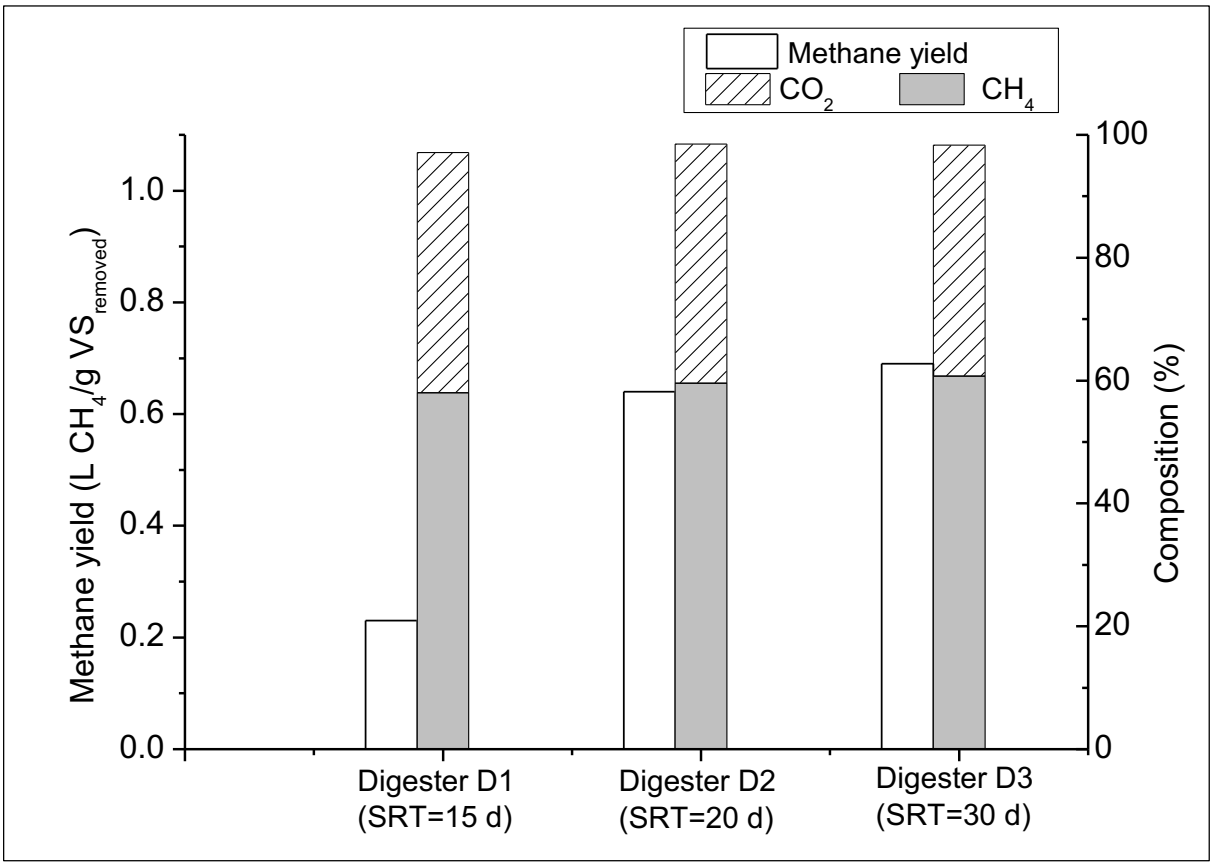


Figure 4.1 Methane yield and biogas composition at SRT of 15, 20, and 30 d.

4.1.2 Sludge characteristic parameters

Primary sludge with TS of 25.7±6.6 g/L was fed to all digester during the experiment, which was in accordance with reported TS range of primary sludge (2.5 - 5.5%) [70]. It should be noted that the TS of primary sludge was subject to the influent of sampling WWTP and weather condition, however, the ratio of VS over TS was relatively stable at 0.89 with minor variation. Corresponding to the observed increase in methane production activity due to increasing SRT, a small nevertheless discernible improvement in the reduction of both TS and VS was observed (Table 4.1). As expected, the reduction of VS was consistently higher than that of TS. As the SRT increased from 15 to 30 days, VS reduction increased from 69.3 to 75.8%. A similar trend was observed regarding the removal of tCOD. tCOD removal increased from roughly 70 to 77% when SRT increased from 15 to 30 d (Table 4.1). On the other hand, the removal of sCOD was not significantly affected by SRT. It should be noted that the soluble COD fraction was relatively small (approximately 2,000 mg/L) compared to the total COD content of the feed (approximately 35,000 mg/L). Overall, results presented in Table 4.1 show significant improvement in basic performance parameters by increasing the SRT beyond 15 days, which

can be attributed to the enhanced methanogenic population and activity at high SRT [22, 103, 224]. On the other hand, the alkalinity at pH=4.5 (Figure 4.2) and pH value of each digester were also monitored weekly throughout the experiment. The values of mixed liquor pH of all three digesters were in the range typical for normal anaerobic digestion (i.e. 7.45 to 7.66). The alkalinity of all digesters was also stable, ranging from 2000 to 3800 mg $CaCO_3$/L (Figure 4.2). Over all, all three digesters were in good condition throughout the experiment period. There was no indication of volatile fatty acid or ammonia accumulation in the digesters.

Table 4.1 Biological performance of three digesters (average ± standard deviation of at least 8 separate samples).

	Digester SRT (d)		
Parameters	15	20	30
TS reduction (%)	59.3±15.0	63.3±14.7	68.6±11.7
VS reduction (%)	69.3±11.8	73.5±12.0	75.8±8.8
tCOD removal (%)	70.2±5.6	71.9±7.8	77.1±5.3
sCOD removal (%)	49.5±18.6	45.8±15.3	53.4±12.1

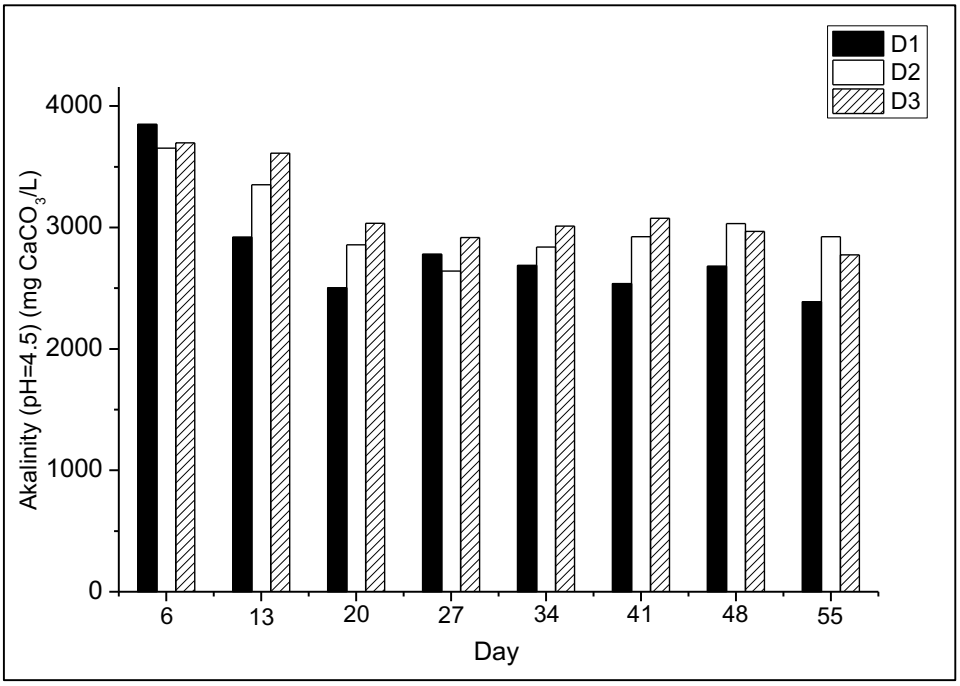


Figure 4.2 Alkalinity of three digesters.

On the other hand, tCOD mass balance of each digester is calculated according to the equation 3.2 – 3.5, and the data is demonstrated in Figure 4.3. As shown in the figure, relatively high COD accumulation was observed in the digester with low SRT (Figure 4.3 a). Meanwhile, increased SRTs reduced the amount of COD contributed to accumulation, and increased the amount of COD converted to methane (Figure 4.3 b and c). It is notable that digester D3 showed negative COD accumulation on sampling day 41, 48 and 55. The reason could be that accumulated COD was determined by the COD input, methane production and COD of digestate (Equation 3-2 – 3-5) on the sampling day, COD samples were taken weekly whilst the COD input from wastewater sludge was varied daily, which may exert instant influence on the COD data on the sampling day. These observations are in accordance with the data presented in Figure 4.1 and Table 4.1 that, higher SRT improved biogas production, TS reduction and tCOD removal. It has been stated from other studies that longer SRT of anaerobic digestion will help to improve sludge reduction and minimize the sludge reproduction [22, 103].

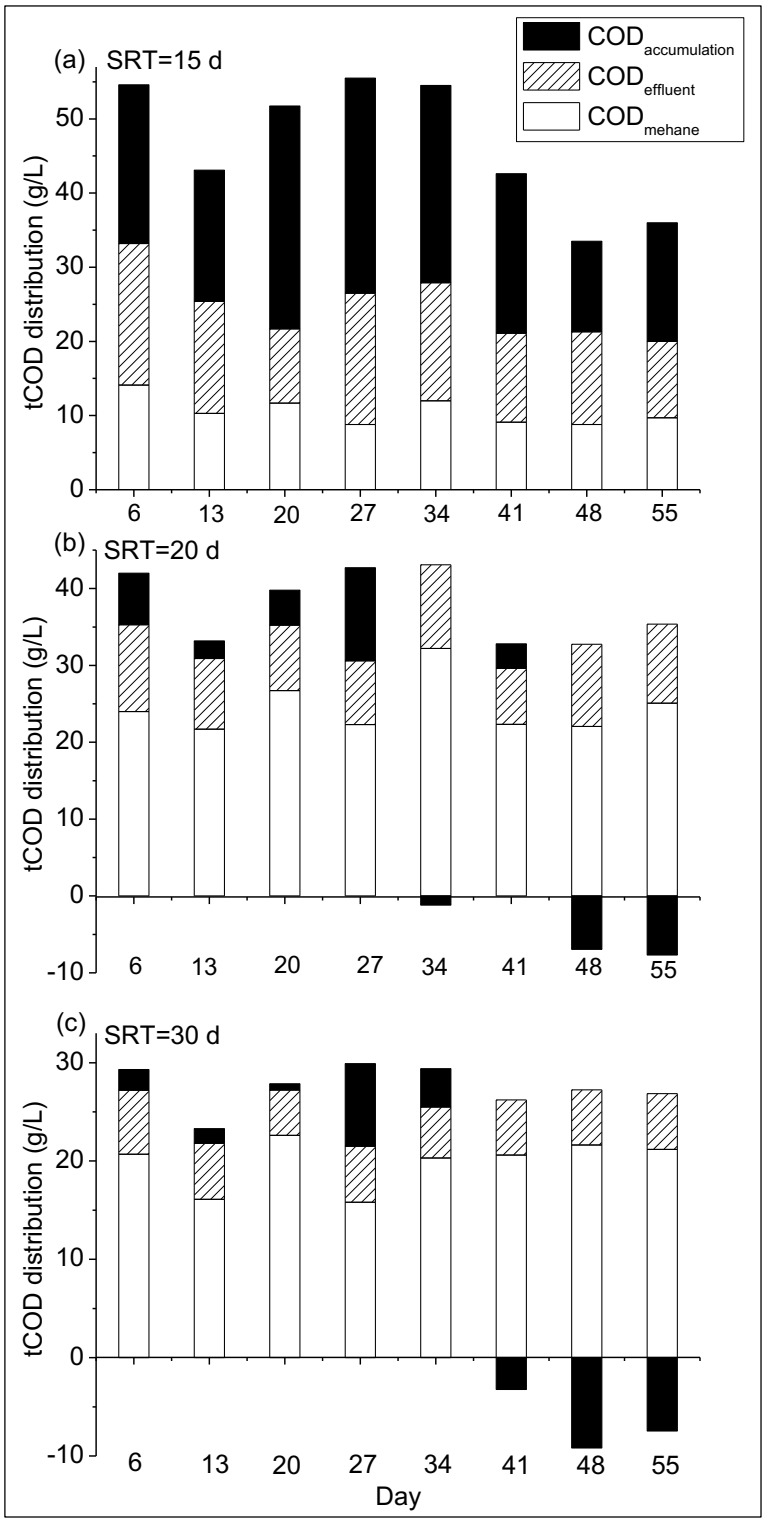
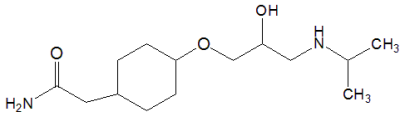
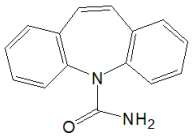
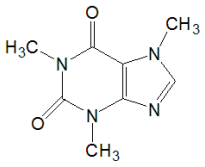
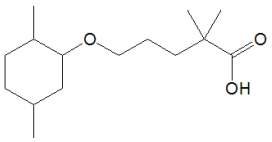
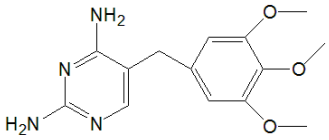
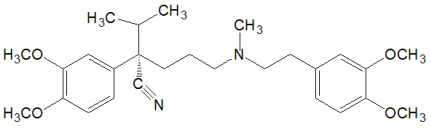
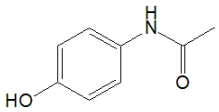
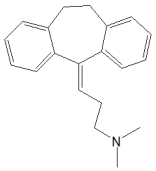
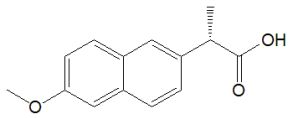
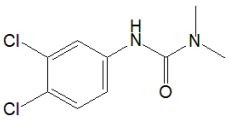
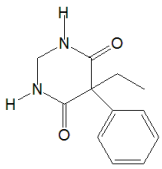
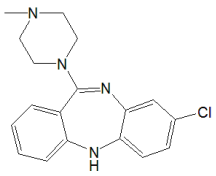
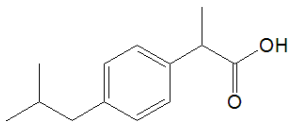
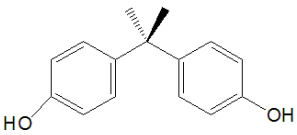
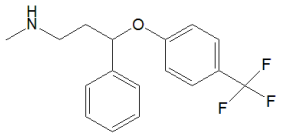
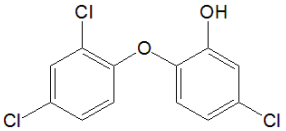
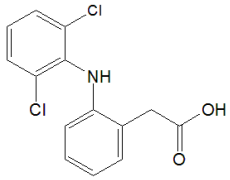
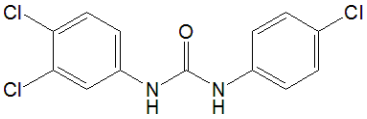


Figure 4.3 COD mass balance for digester (a) D1, (b) D2 and (c) D3 during the experiment.

4.2 TrOC occurrence in the wastewater sludge

During the experimental period, there were 18 TrOCs consistently detected in the primary sludge samples. The compounds and their molecular structure are shown in the Table 4.2.

Table 4.2 Molecular structure of the TrOCs detected in this experiment.

Compounds	Structure	Compounds	Structure
Atenolol		Carbamazepine	
Caffeine		Gemfibrozil	
Trimethoprim		Verapamil	
Paracetamol		Amitriptyline	
Naproxen		Diuron	
Primidone		Clozapine	
Ibuprofen		Bisphenol A	
Fluoxetine		Triclosan	
Diclofenac		Triclocarban	

The concentrations of these TrOCs varied significantly in both aqueous and solid phase of primary sludge. Of these TrOCs, paracetamol, caffeine, ibuprofen and triclosan showed the highest concentrations (>10,000 ng/L) in the aqueous phase (Table 4.3). The prevalent occurrence of these TrOCs in primary sludge can be attributed to their widespread use in our

modern society. Paracetamol and ibuprofen are over-the-counter analgesic and antipyretic drugs. Triclosan is an antibacterial/antifungal agent widely used in soap, detergent, and toothpaste. Caffeine is a stimulant occurring naturally in tea and coffee. Overall, their frequent use in daily life is consistent with the accumulation of these TrOCs in primary sludge [225]. Indeed, antibiotics and pharmaceutically active compounds were amongst the most investigated TrOCs in wastewater sludge in other studies. For example, trimethoprim, sulfamethoxazole were detected at the low mg/kg dry weight range in digested sludge from Swedish wastewater treatment plants [184, 185]; and several compounds like ofloxacin, triclosan and triclocarban exceeded 1000 μg/kg dry sludge in Japan [61]. Several personal care products including triclosan and triclocarban have also been reported to accumulate in the digested sludge to a high concentration after anaerobic digestion [187, 188].

It is notable that all 18 TrOCs detectable in this study occurred predominantly in the solid phase (Table 4.3). In all cases, their concentration in the solid phase (in ng/Kg) was much higher than that in the aqueous phase (in ng/L). The reason could be due to the lipophilicity of the compounds. In other words, they can be transferred to the solid phase during primary and secondary clarification [59], resulting in significantly higher concentrations (several μg/kg dry weight or more) in primary sludge. In addition, the distribution of these TrOCs in the solid phase increased as their logD (the logarithm of distribution coefficient) value increased (Table 4.3). Indeed, for all TrOCs with moderate hydrophobicity (log D > 2 when pH = 5), 72 to 99% of the total mass partitioned in the solid phase (Table 4.3). In line with recent studies concerning anaerobic treatment of wastewater [215, 226, 227], the results here indicated the need to systematically investigate the fate and transport of TrOCs in the liquid and solid phases during anaerobic digestion. During the experiment, there was no additional TrOC spiked to the feed sludge, thus the high standard deviation shown in Table 4.3 also indicates a significant temporal variation in their occurrence in primary sludge. Given the long SRT values (15 to 30 days) used in this study, it was not possible to consider this temporal variation in the feed concentration. Thus, some variation in the calculated removal efficiency would be expected.

Table 4.3 Occurrence of TrOCs of primary sludge in aqueous phase and solid phase (average ± standard deviation of samples taken every 10 days over 12 weeks).

Compounds	log D (pH=5)	log D (pH=7)	Concentration		Mass distribution	
			Aqueous phase (ng/L)	Solid phase (ng/kg dry sludge)	Aqueous phase (%)	Solid phase (%)
Atenolol	-2.75	-2.09	2,649±1,310	94,000±93,000	52	48
Trimethoprim	-1.33	0.27	1,095±263	98,000±67,000	29	71
Caffeine	-0.63	-0.63	50,910±19,50	910,000±497,000	64	36
Paracetamol	0.48	0.47	64,104±52,81	898,000±843,000	71	29
Primidone	0.83	0.83	184±142	22,000±25,000	23	77
Fluoxetine	0.83	1.15	192±102	61,000±31,000	10	90
Clozapine	0.96	3.23	324±97	1,699,000±4,270,	1	99
Verapamil	0.98	2.08	117±38	132,000±69,000	3	97
Amitriptyline	1.35	2.28	791±328	1,023,000±2,398,	3	97
Carbamazepine	1.89	1.89	5,271±1,676	154,000±88,000	56	44
Naproxen	2.49	0.73	2,809±656	23,000±23,000	82	18
Diuron	2.68	2.68	220±47	21,000±12,000	27	73
Ibuprofen	2.81	0.94	12,503±4,716	721,000±1,139,00	40	60
Bisphenol A	3.64	3.64	1,700±1,210	163,000±86,000	27	73
Diclofenac	3.66	1.77	419±217	19,000±16,000	43	57
Gemfibrozil	3.86	2.07	250±124	24,000±13,000	28	72
Triclosan	5.34	5.28	10,680±4,506	1,965,000±1,171,0	16	84
Triclocarban	6.07	6.07	9,212±5,515	4,308,000±1,836,0	7	93

4.3 The fate of TrOCs during the anaerobic digestion

Concentrations of TrOCs in the aqueous and solid phase of primary sludge and digested are shown in Figure 4.4 and Figure 4.5. TrOC removals from both the aqueous and solid phase varied greatly. For example, atenolol, caffeine, trimethoprim, paracetamol and naproxen were well removed from the aqueous phase (Figure 4.4), and they were also effectively removed from the solid phase (Figure 4.5) during anaerobic digestion. On the other hand, several TrOCs including carbamazepine, gemfibrozil, verapamil, amitriptyline, diuron, clozapine, bisphenol A, triclosan, and triclocarbon showed no or only negligible removal from either the aqueous or the solid phase.

It is noteworthy that the pH of the primary sludge was ranging 5.35 to 5.59, while the digested sludge pH was in the range of 7.46 to 7.66. This pH variation may facilitate the transfer of some TrOCs between the aqueous and solid phase when their logD value decreases with increased pH (Table 4.3). Therefore, some compounds were observed higher concentrations in digestate than primary sludge in the aqueous or solid phase. Notable examples include ibuprofen and diclofenac, both of which changed from a moderately hydrophobic to a hydrophilic (increasing solubility in water) form when pH was increased from 5 to 7. As a result, while there was a notable decrease in ibuprofen concentration in the solid phase due to anaerobic digestion, a small but discernible increase in ibuprofen concentration in the aqueous phase can be observed. Meanwhile, the concentration of diclofenac in the aqueous phase increased dramatically after the anaerobic digestion, while the concentration in the solid phase was barely decreased (Figure 4.4 and Figure 4.5).

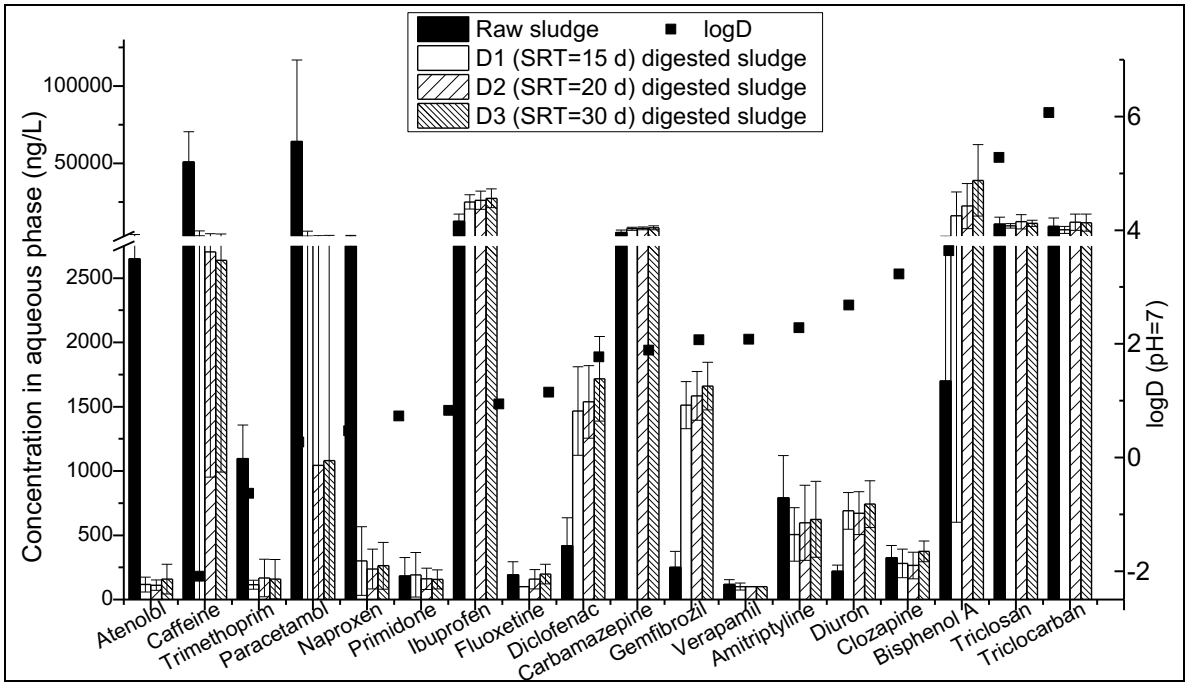


Figure 4.4 Concentration of TrOCs of primary sludge and digested sludge in aqueous phase (error bars show the standard deviation of 12 independent samples).

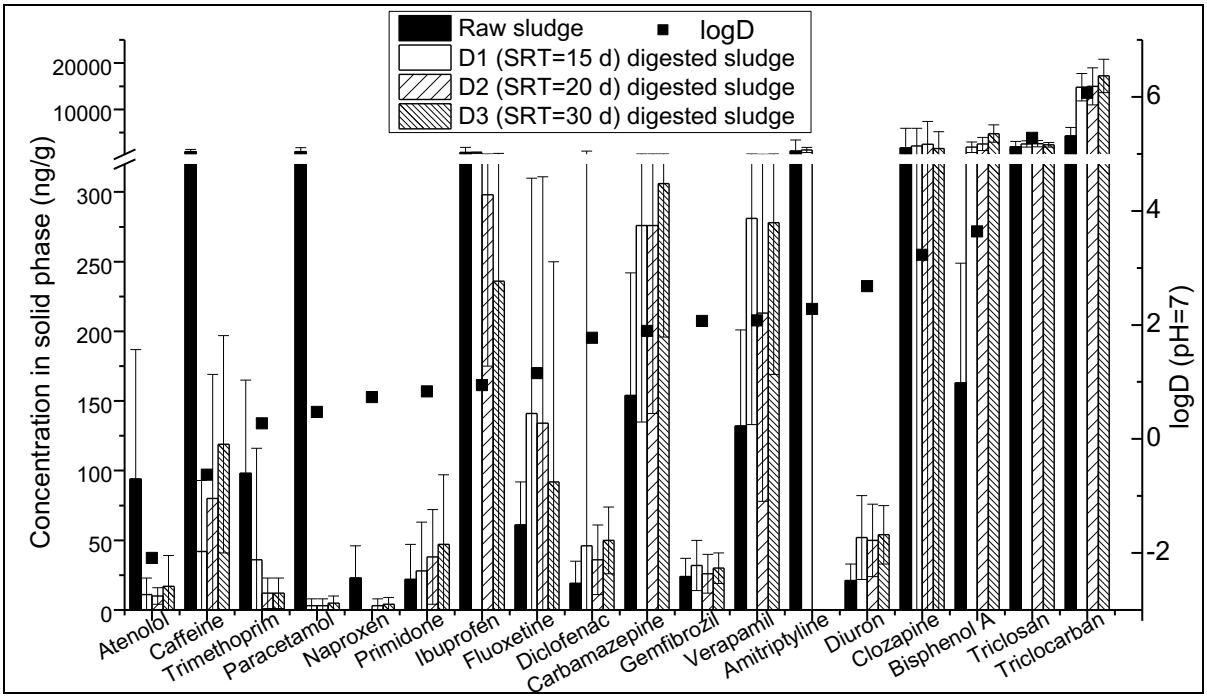


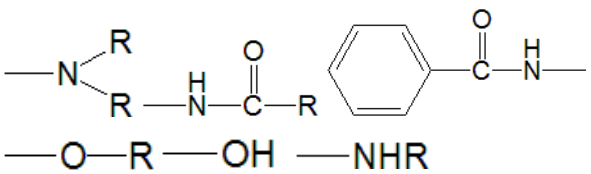
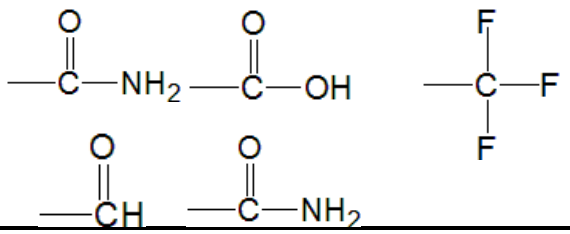
Figure 4.5 Concentration of TrOCs of primary sludge and digested sludge in solid phase (error bars show the standard deviation of 12 independent samples).

On the other hand, the qualitative biodegradation prediction framework proposed by Tadkeaw et al. [228] and recently by Wijekoon et al. [58] for aerobic and anaerobic membrane bioreactors

was used to understand the possible compounds transfer between two phases and biodegradation of each compound. Based on the calculation from Equations 3-6 – 3-8, mass distribution of each compound under different SRTs was presented in Figure 4.6.

The functional groups of TrOCs were found to be the main factor affecting the biodegradation. TrOCs with strong electron donating functional groups were readily degradable under anaerobic condition, leading to high overall removal from anaerobic digestion. Examples of these strong electron donating functional groups are provided in Table 4.4. As a result, atenolol, caffeine, trimethoprim, paracetamol, naproxen, and amitriptyline were well removed by anaerobic digestion (Figure 4.6). On the other hand, TrOCs with strong electron withdrawing functional groups were resistant to anaerobic digestion. Compounds in this group include diclofenac, gemfibrozil, carbamazepine, diuron, and triclocarban given the presence of their chloro and amide moieties which are strong electron withdrawing functional groups (Table 4.4). It is interesting that no removal of bisphenol A was recorded in this study despite the presence of a strong electron donating functional group (hydroxyl). The reason for this observation cannot be confirmed but the release of bisphenol A from plastic component of the experimental system is a plausible explanation. It is interesting to note that TrOCs removal from aqueous phase was reported to be relatively high in previous studies [215, 228, 229], while studies examining the removal of TrOCs from both aqueous and solid phases by anaerobic digestion show that the overall removal efficiency could be much lower [61-63, 197]. The reason could be that most of the hydrophobic compounds would transfer to the solid phase, rather than biodegraded, after the anaerobic digestion. The removal value ignoring the compound residual in the solid phase could not reflect the TrOC's fate during the anaerobic digestion confidently.

Table 4.4 Electron donating and withdrawing functional group found in the TrOCs.

Strong electron donating functional group	Strong electron withdrawing functional group
	

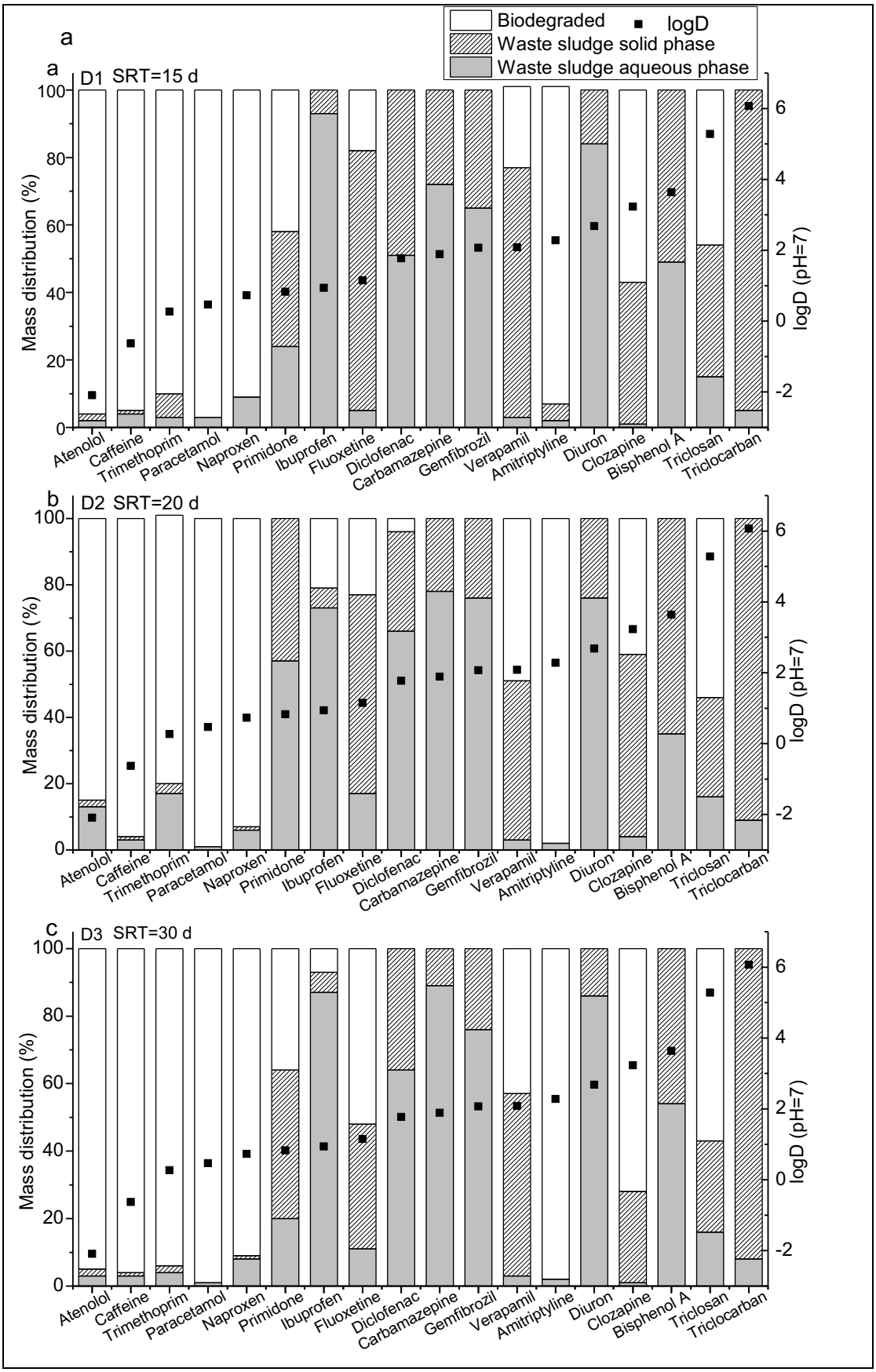


Figure 4.6 Mass distribution of TrOCs after anaerobic digestion at SRT of (a) 15, (b) 20, and (c) 30 days.

There are a few researchers reported the overall removal of the TrOCs during anaerobic digestion. However, the removals of some compounds were controversial. For examples, hormones (estrone, 17β-estradiol and 17α-ethinylestradiol) [62, 230], musk fragrances (galaxolide) [231], diclofenac [61, 62], ibuprofen [62, 231, 232] and triclosan [61, 232] were reported with low or negligible removal; while other studies disagreed. Musk fragrances (galaxolide and tonalide) [63], hormones (estrone, 17β-estradiol and 17α-ethinylestradiol) [63, 232] were removed up to 95% and 75%, respectively. Additionally, compounds like diclofenac [233], trimethoprim [62], diclofenac [61, 233], sulfamethoxazole [61, 232], caffeine [61, 63], naproxen [63, 232], triclosan [233] were well removed by anaerobic digestion. However, the causes of these discrepancies remained unclear.

By comparing the overall removal of each compounds under different SRTs (Figure 4.6), it is shown that no or only a marginal improvement in the removal of TrOCs when the SRT increased from 15 to 30 days. These results were in good agreement with a previous study by Carballa et al. [63] who did not observe any notable increase in the removal of several hydrophilic organic compounds at a prolonged SRT (from 10 d to 30 d). The relative independence between SRT and TrOC removal could be attributed to the fact that they are not the main substrate for the anaerobic digestion process. It is also possible that the improvement in TrOC removal with increasing SRT was not significant and was masked by the variation in feed concentration as discussed in section 4.2.

4.4 Conclusions

Three identical anaerobic digesters were operated under different SRTs (15, 20, 30 d) during this study. The biological performance of the digester was clearly benefited from the increment of SRT that biogas production (0.69 L CH_4/g $VS_{removed}$), TS reduction (68.6±11.7%), VS reduction (75.8±8.8%), tCOD removal (77.1±5.3%) and sCOD (54.3±12.1%) were remarkably improved at SRT of 30 d. At the meantime, TrOCs of primary sludge (feed) and digested sludge were examined to reveal their occurrence and fate during the anaerobic digestion. The significant occurrence of 18 TrOCs in primary sludge was observed. Some of these TrOCs (e.g. paracetamol, caffeine, ibuprofen and triclosan) were also found at very high concentration (>10,000 ng/L) in the aqueous phase probably due to their widespread consumption in society. The overall removal of TrOCs (from both the aqueous and solid phase) by anaerobic digestion was governed by their molecular structure (e.g. the presence/absence of electron

withdrawing/donating functional groups). The lack of influence of SRT on TrOC removal suggests that TrOCs were not the main substrate for the anaerobic digestion process.

Chapter 5 Improvements to anaerobic digestion by recuperative thickening

In recent years, urbanization and population growth have exerted an event greater treatment capacity demand on existing WWTPs and waste management facilities. However, in many cases, due to space limitation, expansion of the physical footprint of the treatment plant is simply not possible. As a result, several techniques to increase the treatment capacity without the need for additional space have been proposed and investigated. One of them is recuperative thickening, which was first introduced in 1967 [35]. As reviewed in Chapter 2, recuperative thickening is a modified anaerobic digestion process, which can extend SRT from HRT. Recuperative thickening has been approved to improve the organic conversion to methane gas and increase the treatment capacity without reducing SRT [67, 209]. On one hand, SRT extension improves the conversion of organics to methane and increase the volatile solid (VS) reduction [38, 67]; on the other hand, recuperative thickening increases the solids concentration to dewatering, which has been shown to increase sludge dewater ability [67]. In this chapter, three identical lab-scale anaerobic digesters were used to study the impact of recuperative thickening on the biogas production, TS and VS reduction, COD removal, dewatering ability and odour components of dewatered sludge cake etc.

Three sets of experiments were conducted to achieve different SRTs and HRTs when recuperative thickening was applied. In all experiments, digester D1 was operated as a control system without recuperative thickening, and digester D2 and D3 were operated with recuperative thickening to achieve higher SRTs. The detailed experiment plan is listed in Table 5.1. Dewatering polymer (Zetag® 8165, BASF) was applied to the digested at concentration of 7.5 g/kg dry sludge to separate solid and supernatant, and thickened sludge was then returned to the digester with primary sludge. During the experiments, anaerobic digesters performance parameters, including biogas production and composition, TS, VS, COD, alkalinity pH etc, were monitored regularly, and at the end of experiment dewatered sludge cake was prepared to exam the dewaterability and odour components. The following section will present and discuss the experimental results.

Table 5.1 Experimental plan for anaerobic digesters with recuperative thickening.

	Experiment 1			Experiment 2			Experiment 3		
	D1	D2	D3	D1	D2	D3	D1	D2	D3
Active volume (L)	20			20			20		
Recuperative thickening	No	Yes	Yes	No	Yes	Yes	No	Yes	Yes
HRT (d)	20			20			10		
SRT (d)	20	25	30	20	30	60	10	15	20
Thicken ratio	None	1.2	1.33	None	1.33	1.67	None	1.67	2
Primary sludge (L/d)	1			1			2		

5.1 Biogas production and composition

Biogas production rates of all three digesters were monitored by the biogas counter continuously in experiments. As elucidated in Figure 5.1, decoupling of SRT from HRT by recuperative thickening has resulted in a notable increase in biogas production, particularly when SRT increased from 15 to 20 day with control system SRT of 10 d (Figure 5.1c). By contrast, recuperative thickening only led to slight improvement of biogas production when SRT of control digester was 20 d (Figure 5.1a and b). The digesters operated under SRT of 25 d (D2 in Figure 5.1a), 30 d (D3 in Figure 5.1b and D2 in Figure 5.1c) and 60 d (D3 in Figure 5.1c) produced similar or slightly more biogas comparing to the control system. These results suggested that SRT between 20 and 30 d could be optimal for anaerobic digestion in terms of biogas production [9, 24], and further increment of SRT did not enlarge the biogas production.

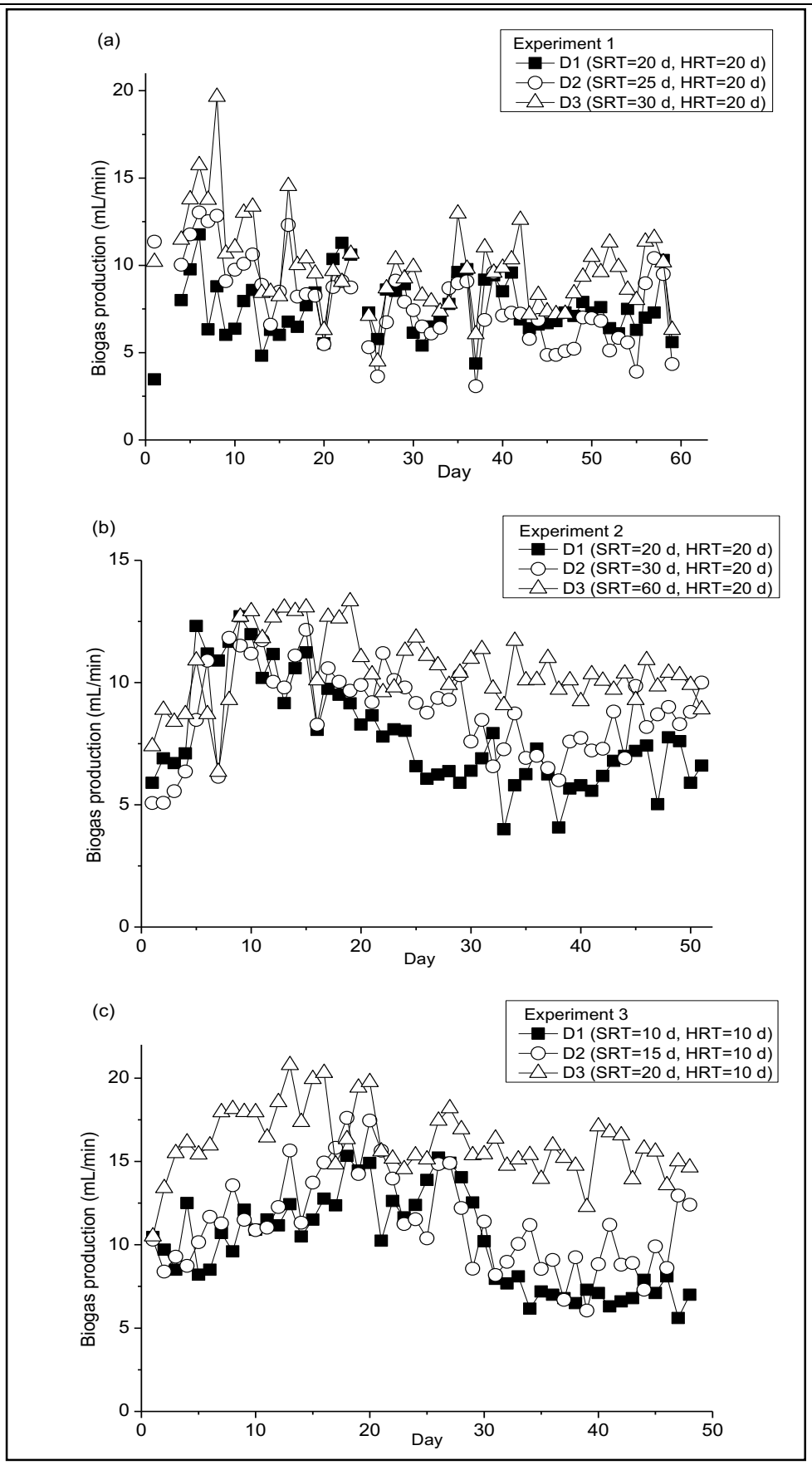


Figure 5.1 Biogas production rates of all digesters (experimental conditions are noted in Table 5.1).

Recuperative thickening also resulted in a notable increase in the methane yield which is defined as methane production per gram of VS removed (Figure 5.2). Recuperative thickening possibly enriched methanogenic bacteria, allowing for enhance methane transformation from organic fractions. It is shown in Figure 5.2 that highest methane yield occurred when HRT was 20 d and SRT was 30 d with recuperative thickening (Experiment 1); however, further increase of SRT to 60 d by recuperative thickening did not enhance the methane production activity (Experiment 2). It is notable that increment of SRT by recuperative thickening at low HRT (10 d) only slightly improved the methane yield; however, all digesters did not achieve relatively high methane production activity during experiment due to low HRT. The result indicates that optimum SRT and HRT for this anaerobic digestion system could be 30 d and 20 d, respectively; and recuperative thickening would be an effective way to achieve such retention times.

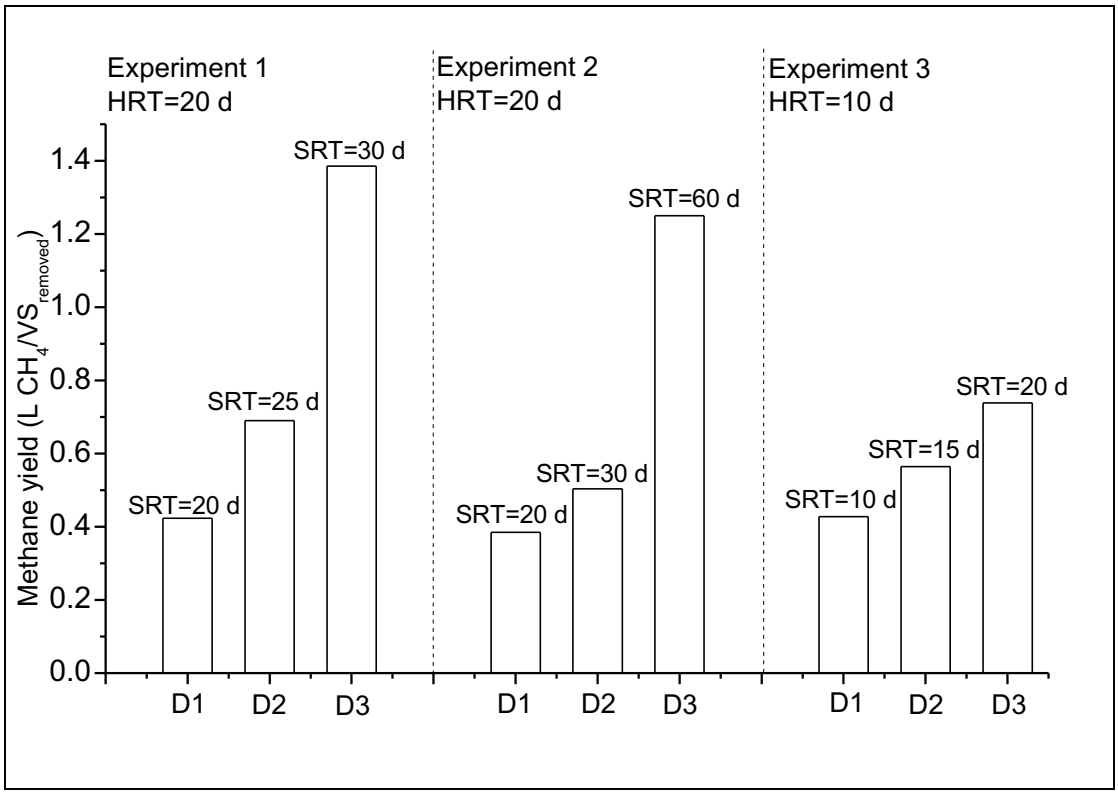


Figure 5.2 Methane yield of individual digester during the experiments.

On the other hand, the composition of the biogas is also an important indicator for anaerobic digester performance and stability. In all experiments (Table 5.2), the biogas composition was stable and consistent with values reported in the literature [234, 235], in which approximately 60% of CH_4 and 40% of CO_2 were observed. Additionally, trace of H_2S and O_2 were also detected in the gas samples that up to 25 ppm of H_2S and 2.1% of O_2 were observed. It is important to note that oxygen could be introduced to the digester during the thickening and sludge recycling processes; however, no deleterious effects due to possible air exposure were

observed in the digesters in these experiments. It has been stated by other studies that low level of oxygen exposure during thickening process (such as gravity belt) had no apparent effect on the methane production [40, 210]. The reason methanogenic bacteria can be tolerate to low range oxygen exposure is that methanogenic bacteria are well protected in sludge granules, and oxygen-consuming facultative bacteria in the immobilised consortia can metabolise part of the available substrate and consume oxygen, creating anaerobic microenvironments [210].

Table 5.2 Composition of biogas samples from digesters during the experiments (Data show mean ± standard deviations of 6-8 measurements).

	Digester	HRT (d)	SRT (d)	CH_4 (%)	CO_2 (%)
Experiment 1	D1	20	20	56.9±3.0	39.0±3.5
	D2		25	60.2±1.1	37.2±1.4
	D3		30	59.2±0.8	37.0±1.0
Experiment 2	D1	20	20	58.1±1.0	37.7±1.0
	D2		30	58.7±0.3	38.7±0.6
	D3		60	57.9±1.0	38.6±1.3
Experiment 3	D1	10	10	59.1±2.2	37.3±1.1
	D2		15	61.1±4.1	36.3±2.4
	D3		20	59.6±2.4	37.5±0.6

5.2 Sludge character and digester stability

All three digesters were fed with primary sludge sampled from full-scale wastewater treatment plant. Therefore, the TS and VS of the primary sludge are weather dependent and thus can vary. In this study, TS of the primary sludge was 24.9±5.1g/L (mean ± standard deviations of 24 samples). Similar temporal variation could also be observed with the tCOD of primary sludge. Despite this variation in the TS content, VS/TS ratio of the raw primary sludge remained relatively constant at 0.85±0.06 (mean ± standard deviations of 24 samples).

5.2.1 TS, VS and COD of digested sludge and their removals

Throughout the experimental period, sludge characters such as TS, VS, tCOD and sCOD were measured for digested sludge and primary sludge samples. It is important to note that solid (TS and VS) and tCOD value of digesters with recuperative thickening were remarkable increased due to returning thicken digested sludge to the digester (Table 5.3), therefore their removals could be affected by the thicken ratio when comparing to the same feed inlet of control system (primary sludge). It is notable that although solid related parameters such as TS, VS and tCOD were majorly increased by thickening process compared to control system, sCOD values were barely impacted by recuperative thickening (Table 5.3)

Table 5.3 Average TS, VS, tCOD and sCOD of each digester during the experiments (mean± standard deviations of 8 measurements).

	Experiment 1			Experiment 2			Experiment 3		
	D1	D2	D3	D1	D2	D3	D1	D2	D3
Recuperative thickening	No	Yes	Yes	No	Yes	Yes	No	Yes	Yes
TS (g/L)	8.53±2.28	13.20±2.5	14.63±4.21	8.10±1.60	10.05±4.35	16.38±9.16	10.30±1.91	12.96±2.00	12.55±2.02
VS (g/L)	5.88±1.79	9.03±2.03	9.94±2.52	5.39±1.07	6.73±2.47	10.74±5.27	7.22±1.29	9.37±1.62	9.06±1.55
tCOD (mg/L)	13713±4600	15300±3768	41375±70949	10013±3440	10338±3789	15488±4408	12300±1989	15175±6750	14325±2983
sCOD (mg/L)	923±382	736±317	896±466	633±242	650±139	484±230	1140±1042	750±81	668±146

In order to eliminate the influences of thicken ratio caused by recuperate thickening, the TS, VS, tCOD and sCOD of digested with recuperative thickening were normalised by the thicken ratios (Table 5.1) respectively. The relative removals of these digesters were then calculated by the normalised value accordingly. Figure 5.3 – Figure 5.6 demonstrates the TS, VS, tCOD and sCOD removals of control system; on contrast, the relative removals of above parameters were shown for D2 and D3 during all 3 experiments.

Considerable variation in the removal/relative removal of TS, VS, tCOD and sCOD over time was observed (Figure 5.3 - Figure 5.6). The standard deviation of TS, VS and tCOD removal over time was up to 11%. This variation was attributed to the temporal variation in the primary sludge as discussed above. During the experiment 1 and 2 when HRT was 20 d, the TS, VS, tCOD removals of digesters with recuperative thickening (D2 and D3) were not observed with significant improvement, even when the SRT was extended to 60 d. On contrast, increased SRT when HRT was 10 d had led to evident increment of TS, VS and tCOD removals in experiment 3. These results provided additional experimental evidence to previous findings by Reynolds et al. [37] and Ostapczuk et al. [212]. These authors reported that recuperative thickening would be ineffective to remove VS in full-scale digesters if adequate SRT value had been achieved. The finding reported in our study and those by Reynolds et al. [37] and Ostapczuk et al. [212] can be attributed to several factors. For instance, recuperative thickening resulted in an increase in the organic loading rate. Such an increase in organic loading rate could decrease the COD removal in the digester [26, 27]. In addition, the improvement in system stability could be masked by the variation in the characteristics of the primary sludge and the build-up of less biodegradable volatile solids in the digester. It is known that the organic content of primary sludge is heterogeneous. In other words, primary sludge contains organic fractions with different degrees of biodegradability. At a sufficiently high baseline SRT value, the returned thickened sludge due to recuperative thickening would contain mostly organic matter that is rather recalcitrant to further biodegradation. The results from this study suggested that organic matter destruction (VS or tCOD) was not significantly improved by recuperate thickening in experiment 1 and 2, because the solids returned to the anaerobic digester have a smaller proportion of readily biodegradable materials at SRT greater than 20 d.

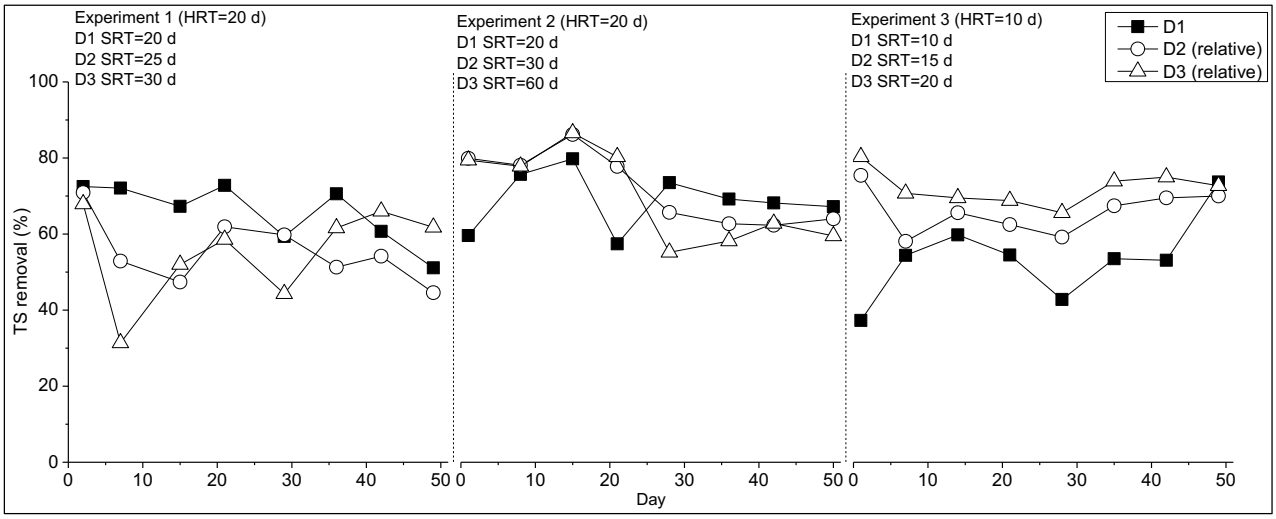


Figure 5.3 TS removals/relative removals for digesters during Experiments 1-3.

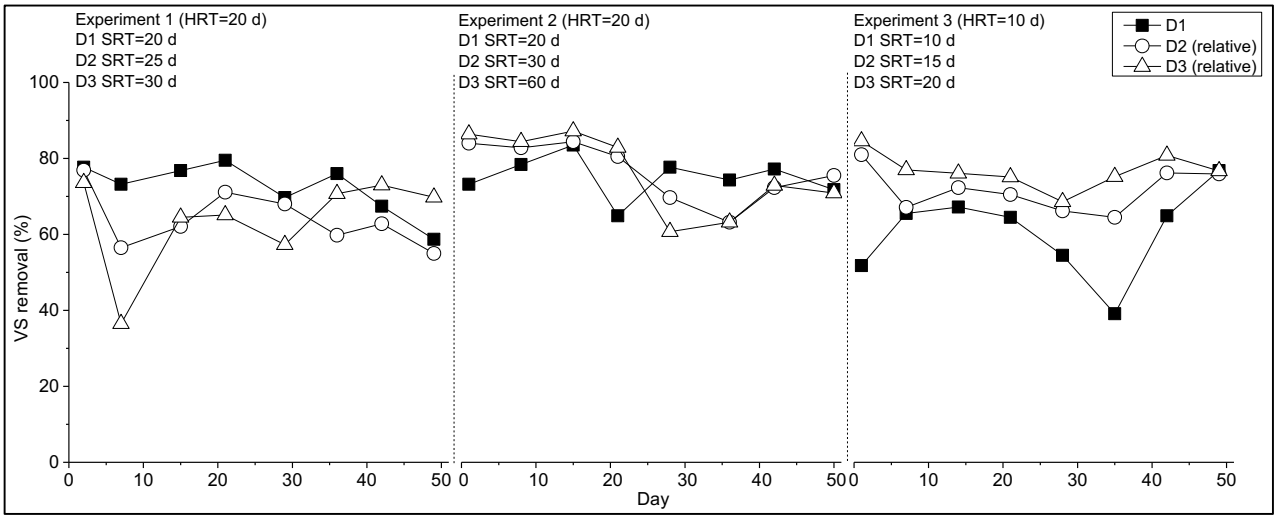


Figure 5.4 VS removals/relative removals for digesters during Experiments 1-3.

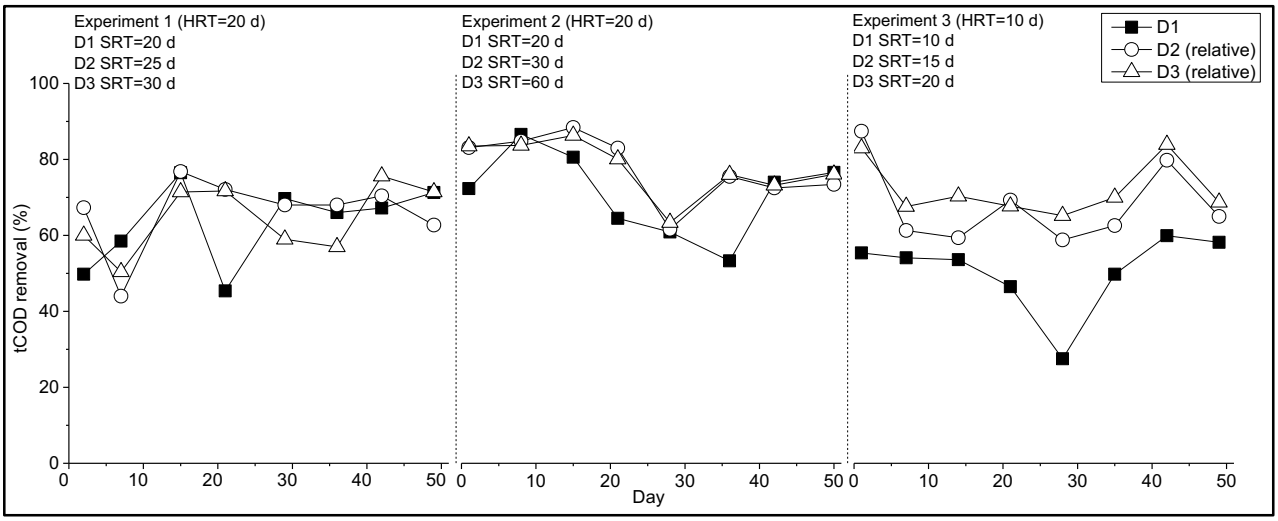


Figure 5.5 tCOD removals/relative removals for digesters during Experiments 1-3.

The removal of sCOD (Figure 5.6) was improved in all experiments by recuperative thickening in comparison to the control (digester D1). This can also explain the higher biogas production without discernible improvement in the removal of tCOD with recuperative thickening. This can be attributed to the sequestration of soluble and biodegradable macromolecules and colloidal particles from the aqueous solution into the solid phase caused by polymer addition during thickening. In other words, the addition of polymeric to the sludge prior to thickening would allow for some soluble macromolecules and colloidal materials to be captured and returned to the digester. Although sCOD (approximately 1000 mg/L) only contributed to less than 5% of the total COD content of raw primary sludge, the improvement in sCOD removal could also be a factor attributing to the higher biogas production due to recuperative thickening.

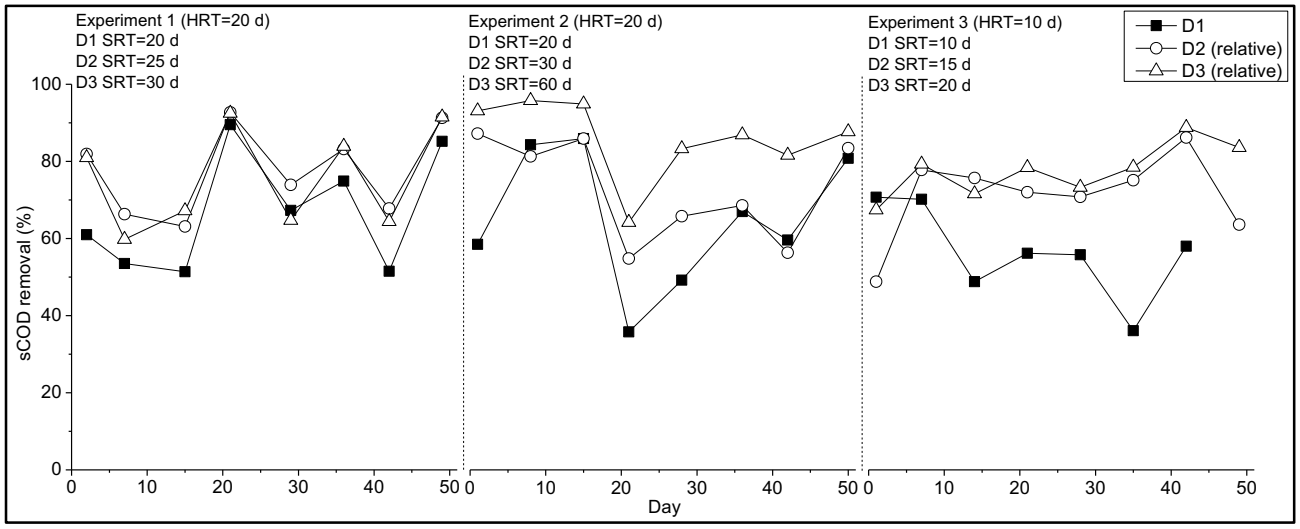


Figure 5.6 sCOD removals/relative removals for digesters during Experiments 1-3.

The results demonstrate that recuperative thickening could be used for plants with inadequate HRT (i.e. < 15 d) to improve the removal of VS and tCOD. Our results highlight the role of recuperative thickening in decoupling SRT from HRT thus allowing for the increase in SRT and improve system stability through the reduction of short circuiting and alleviating the impact of feed variation.

5.2.2 Digester stability indicators

It is important to note that all digesters performed stably throughout all experiments periods, although the TS, VS and COD of feed (primary sludge) varied. As the indicators for the digester stability, pH and alkalinity of each digester were monitored all the time. pH values of all digesters were between 7.2 – 7.8 throughout all experiments. On the other hand, alkalinity was over 2000 mg $CaCO_3$/L for all digesters, with only one exception at 10d SRT (Figure 5.7). The

results confirm that exposure of the thickened sludge to air did not negatively affect anaerobic performance. This is consistent with previous studies by Conklin et al. [40] and Reynolds et al. [37] who studied the effect of short-term oxygen exposure to anaerobic digester sludge in batch mode to simulate recuperative thickening condition.

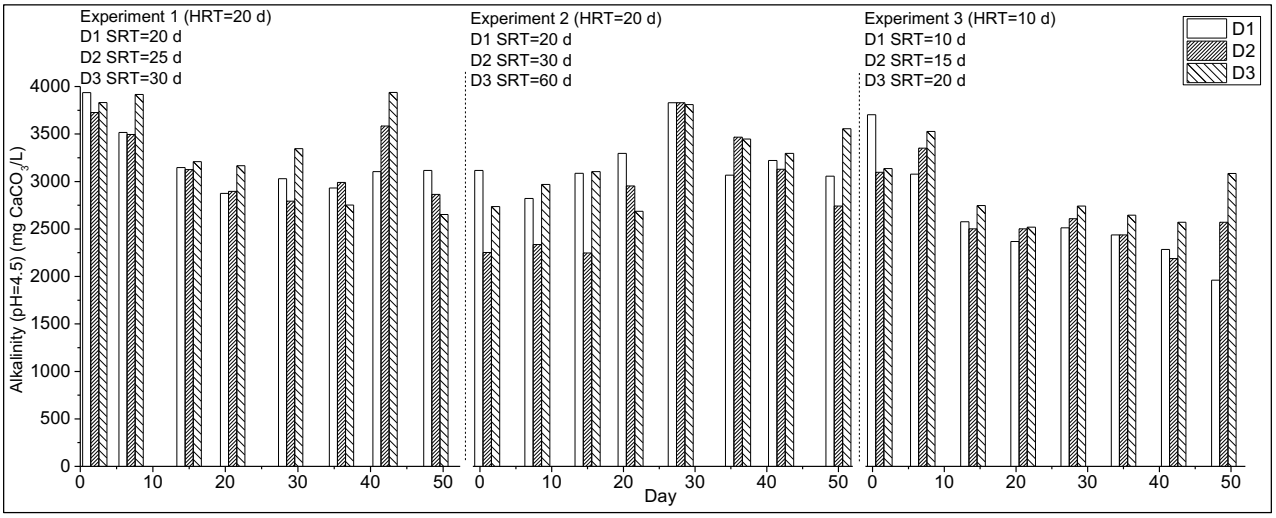


Figure 5.7 Alkalinity of digesters during Experiments 1-3.

5.2.3 Mass balance (tCOD) for digesters

To further elucidate the build-up of less biodegradable organic matter in the digester at high SRT and the associated influence on COD removal, a mass balance with respect to COD was calculated for all experiments according to the Equation 3.2 – 3.5 (Figure 5.8). Each bar in Figure 5.8 presents the total COD input of the sampling date. The COD accumulated in the digested sludge is calculated as the balance of the COD input and output. Mass balance analysis showed that recuperative thickening resulted in higher conversion of COD to biogas. However, both digesters D2 and D3 (compared with digester D1) had more COD accumulated throughout the experimental period (Figure 5.8). This explains the insignificant improvement in COD removal due to recuperative thickening when SRT of the digester D1 (control without recuperative thickening) was sufficiently high (20 d). Thus, recuperative thickening resulted in an increase in not only the COD consumed for biogas production but also COD accumulation in the digester.

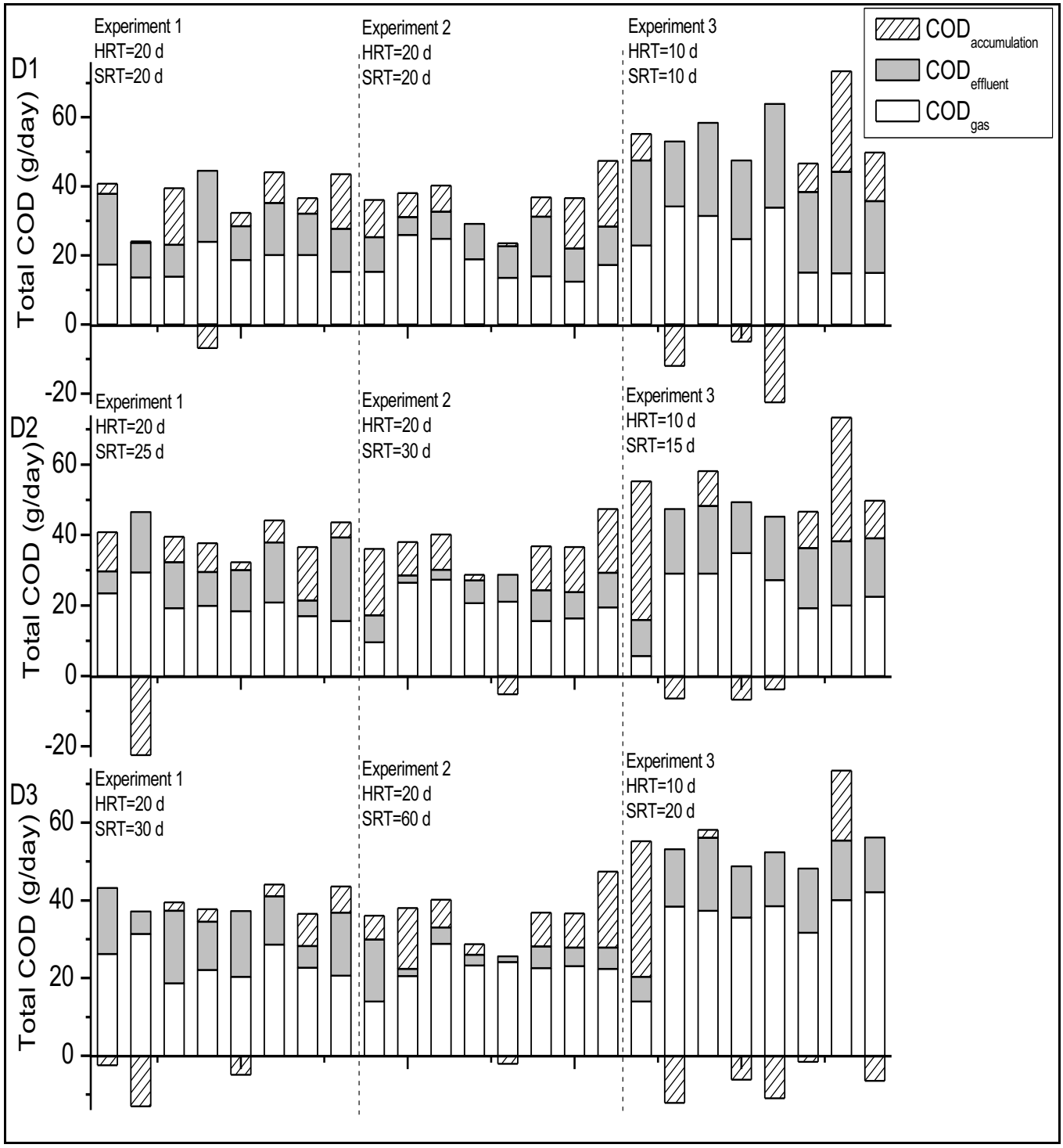


Figure 5.8 Total COD balance for each digester during the experiments.

5.3 Digested sludge dewatering and the odour compounds in the dewatered sludge

5.3.1 Sludge dewatering

At the end of each experiment, duplicated sludge samples from each digester were taken to prepare dewatered sludge cake by the method described in Chapter 3, section 3.3.3. The dry solid content of the biosolid cake is an indicator for sludge dewaterability. Certain amount of the biosolid cake (60 g) was stored in a sealed bottle (250 mL), and then incubated at 25 °C. The air from bottle head space was taken to analyse the concentration of volatile organic sulphur compounds in the dewatered sludge cake. Table 5.4 lists the average dry solid content of each digester's biosolid cake after dewatering. The data showed that biosoild cake from digesters with recuperative thickening (D2 and D3) had higher dry solid content than control (D1); however, dry solid content of biosolids from D2 and D3 in different experiments had no significant differences. This observation proved that recuperative thickening could improve the digested sludge dewaterability compared to control; however, further SRT increment by recuperative thickening did not benefit the dewaterability in all experiments.

It has been stated that extracellular polymeric substances (EPS) play significant role on bioflocculation and dewatering of sludge. EPS was approved to be essential to sludge-floc formation [236, 237], however, excessive EPS could lead to limited dwaterability due to weakened cell attachment and floc structure [237]. On the other hand, the behaviour of a molecule of water during the dewatering process was depended on its proximity to the solid [238]. Bound water could be released and converted into free water by degradation of EPS [239] or polymer conditioning [240], thus, mechanical dewatering would be improved. Ahn et al. [241] had found that increase of SRT from 15 d to 20 d led to decreased total soluble EPS, which resulting in higher settling rate of microorganisms and better dewaterability. Similar observation was made by Liu and Fang [242], who reported that increase of EPS in sludge would lower sludge dewaterability. It is important to note that anaerobic digested sludge had lower sludge dewaterability compared to activated sludge, thus, a dosage of ployelectrolyte conditioner is required [240, 242].

Table 5.4 Dry solid content of biosolid cake (mean±standard deviations of 2 samples).

Digester	Dry solid content (%)		
	Experiment 1	Experiment 2	Experiment 3
D1 (no recuperative thickening)	14.5±1.8	17.1±0.9	17.6±1.6
D2 (recuperative thickening)	24.1±3.1	21.6±1.3	22.6±2.0
D3 (recuperative thickening)	23.3±1.7	23.1±2.4	22.2±1.3

5.3.2 Odour compounds concentration of dewatered sludge

As mentioned in section 3.3.3, dewatered sludge cakes were samples and incubated in order to analysis the volatile organic sulphur compounds (VOSCs) concentration in the biosolids. Dewatered sludge cake was incubated for 7 days at 25 ºC, and headspace gas was sampled at day 1, 3 and 7. There were 7 VOSCs tested, namely hydrogen sulphide, methyl mercaptan, ethyl mercaptan, dimethyl disulphide, carbon disulphide, dimethyl sulphide, carbonyl sulphide. Among them, hydrogen sulphide, methyl mercaptan and carbonyl sulphide were the most abundant compounds, and their concentrations for biosolid cake from each digester in experiment 3 were demonstrated in Figure 5.9

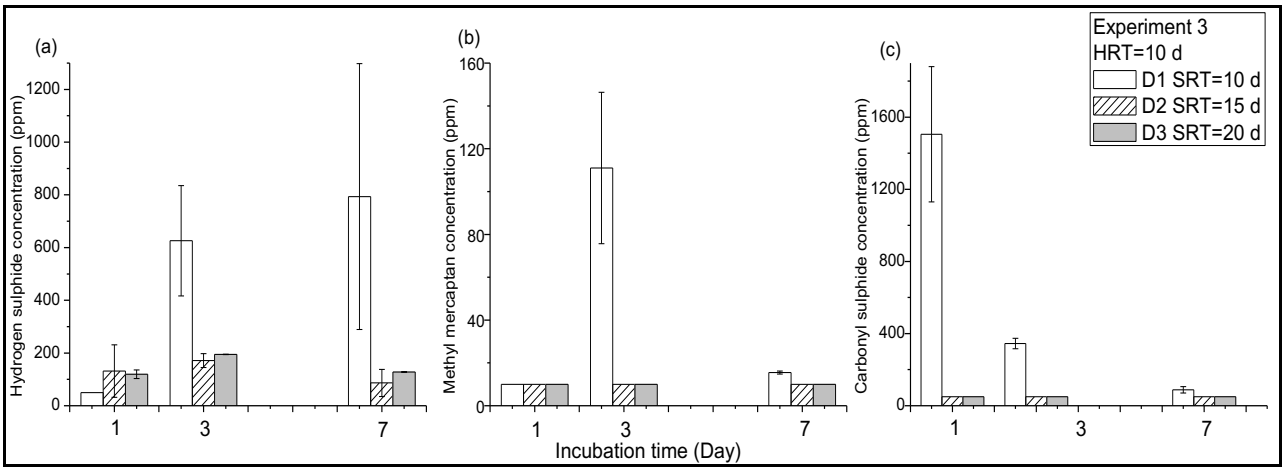


Figure 5.9 Concentration of hydrogen sulphide (a), methyl mercaptan (b) and carbonyl sulphide from biosolids of experiment 3 as a function of incubation time.

Hydrogen sulphide was prevalent in all biosolids cake samples, and methyl mercaptan (which is one of the more important compounds for malodour reception associated with biosolids) and

carbonyl sulphide could also be detected from biosolids cake samples (Figure 5.9). Biosolids cake from D1 (which was operated at SRT of 10 d without recuperative thickening) exhibited significant higher concentration of all three odour compounds compared to both digesters D2 and D3 which were operated with recuperative thickening at SRT of 15 and 20 d, respectively. Additionally, the concentration of total volatile organic sulphur compound also reduced, indicating that recuperative thickening reduces malodour generation associated with the biosolids cake. Results reported in this study show, for the first time, that recuperative thickening can contribute to a notable reduction in volatile organic sulphur compounds possibly due to enhanced organic matter destruction, and hence, in biosolids cake odour.

5.4 Conclusions

Recuperative thickening was a modified anaerobic digestion process which could extend SRT from HRT. In this study, three different sets of experiments were conducted to study the effect of recuperative thickening on the anaerobic digestion. Compared to the digester with accordant SRT and HRT (20 d), recuperative thickening led to doubled methane production (approximately 1.37 L CH_4/g $VS_{removed}$) as well as system stability when SRT and HRT were 30 d and 20 d, respectively, which could due to the increment in SRT and a reduction in short circuiting. There was also evidence that improved performance associated with recuperative thickening would be due to sequestration of biodegradable and soluble macromolecules and colloidal particles promoted by polymer addition used to thicken the sludge as well as possible enrichment of methanogens in the digesters. However, the removals of TS, VS and tCOD were not significantly improved by recuperative thickening when baseline SRT was 20 d, indicating that recuperative thickening did not enhance the organic matter destruction at a sufficiently high baseline SRT value. Thus, recuperative thickening could be used for plants with inadequate HRT (i.e. < 15 d) to improve the removal of VS and tCOD. Recuperative thickening also led to improved sludge dewaterability and a reduction in total volatile organic sulphur compounds. This would result in the production of less odorous biosolids. Results reported in this study indicate that RT would be a viable technique to improve the performance of anaerobic digesters with inadequate SRT or issues with system stability. The results obtained in this study need validation by a full-scale investigation.

Chapter 6 Impacts of sheared thickening process on the anaerobic digester performance, microbial community structure and TrOC removal

As reviewed in Chapter 2, recuperative thickening is a feasible solution to enlarge SRT and treatment capacity of anaerobic digestion without the need for additional space. It has been stated in Chapter 5 that recuperative thickening could be used for plants with inadequate HRT (i.e. < 15 d) to improve the removal of VS and tCOD. However, thickening process would inevitably influent anaerobic digestion performance by introducing oxygen exposure and shear force to sludge. As discussed in Chapter 2, the effect of oxygen exposure on the methanogenic activity is negligible during some thickening processes such as gravity belt and centrifuge, because of methanogens' tolerance to low range of oxygen exposure. On the hand, during the thickening process by a centrifuge or rotary drum, the sludge is subjected to shearing. Recent investigations of recuperative operation and sludge thickening have focused most only on the methane production although there is some evidence that sludge shearing may also affect the microbial structure and thus methanogenic activities. Thickening process by centrifuge has been reported to be effective to increase methane production in both lab-scale digester [41] and full-scale wastewater treatment plants [42, 43]. Nevertheless, high speed centrifuge [44] or high shear forces (2.01–3.35 m/s) [46] were observed to cause the loss in the viability of bacterial population. Little is known about the effects of different levels of shearing of the thickened sludge on biogas production and the microbial community. Thus, this study aims to quantify the effects of shearing during recuperative thickening on biogas production as well as COD and VS removal by anaerobic digestion. The microbial community structure of the digested sludge is also systematically examined to elucidate dynamic changes in microbial community in response to shearing. Meanwhile, TrOC removals of digesters with different shearing level were also examined.

All three digesters were operated with recuperative thickening to achieve an SRT of 30 days while maintaining an HRT value of 20 day. Each day, 2 L of sludge was extracted from the digester, and 0.67 L was wasted. Thickening polymer (Zetag 8169, BASF) was added to the remaining digested sludge at a dose of 7.5 g/Kg dry sludge for thickening. This high and conservative polymer dose is to ensure consistent thickening regardless of the shearing

condition. Supernatant was then discarded and 1 L of the thickened sludge was returned to the digester together with the daily feed (i.e. 1 L of primary sludge).

Digester D1 was the control system with gravity thickening (designated as no shearing) during the thickening process. Shearing was applied to thickened sludge from digesters D2 and D3 (Table 6.1). An agitator (Servodyne mixer head, model 50003-25, Boronia, Australia) with a 2-blade bending paddle impeller (5 cm x 10 cm) was used to provide medium shearing at 300 rpm (G = 3140 s^{-1} comparable to the shearing level of a typical rotary drum) and high shearing at 600 rpm (G = 6280 s^{-1} comparable to the shearing level of a typical high speed centrifuge) to the thickened sludge from D2 and D3, respectively. A food blender (Sunbeam, model PB9500, Australia) was also used to simulate excessive shearing to the thickened sludge from digester D3 (Table 6.1). In all cases, the shearing process lasted 5 minutes.

Digester D3 was regenerated at the end of second experimental period by renewing part of its sludge. 5 L of the digested sludge from D3 was replaced by freshly collected anaerobically digested sludge from wastewater treatment plant (Period 3), and another 5 L of the digested sludge was replaced again in 2 weeks' time (Period 4). During these 2 periods, no shearing was applied to thickened sludge from D3.

Table 6.1 Experiment regimes for anaerobic digestion with sheared thickening process.

Operational parameters	Period 1 (Day 1-55)			Period 2 (Day 56-114)			Period 3 & 4 (Day 115-142)		
	D1	D2	D3	D1	D2	D3	D1	D2	D3
Recuperative		Yes			Yes			Yes	
Thicken ratio		1.33			1.33			1.33	
HRT (d)		20			20			20	
SRT (d)		30			30			30	
Shearing (level)	None (None)	300 rpm (Medium)	Blender (1000 rpm) (Excessive)	None (None)	300 rpm (Medium)	600 rpm (High)	None (None)	300 rpm (Medium)	None (None)
G value	N.A.	3140 s^{-1}	<6280 s^{-1}	N.A.	3140 s^{-1}	6280 s^{-1}	N.A.	3140 s^{-1}	N.A.

Anaerobic digester performance was monitored according to the methods mentioned in Chapter 3, and additionally sludge was taken to prepare TrOCs samples. At the end of period 1 (day 55)

and period 2 (day 110), digested sample from each digester were taken for DNA extraction and 16S rRNA gene amplicon sequencing in order to analyse the microbial community structure.

6.1 Anaerobic digester performance

6.1.1 Biogas production and composition analysis

There were some discernible effects of shearing on biogas production during recuperative thickening (Figure 6.1). Compared to the control digester (D1), digester D2 produced approximately 15% more biogas throughout the experiment periods (Figure 6.1), which is comparable to the 10% increase in biogas yield observed by [39] when they conducted recuperative thickening experiment without any shearing. This is consistent with full scale observation by Bharambe et al., [68] in which sludge thickening was achieved by a rotary drum. In our study, the thickened sludge that was circulated back to digester D2 was also subjected to medium shearing (equivalent to that from a rotary drum). On the other hand, excessive shearing (by a food blender) was detrimental to biogas production. Biogas production from digester D3 was approximately 30% lower than that of the control digester (D1) in the 1st experimental phase. The level of shearing applied to the thickened sludge of digester D3 was induced by a mixer at 600 rpm (equivalent to that from a high-speed centrifuge) rather than the food blender in period 2; however, improvement in biogas production could not be observed (Figure 6.1).

Similar trends were observed when examining the methane production activity (Table 6.2). Methane production activity of D2 (approximately 0.5 L CH_4/g CODremoved) was similar to that of the control system D1 throughout experiment periods 1 and 2, and gradually increased to approximately 0.73 L CH_4/g $COD_{removed}$ at the end of the experiment (period 4). By contrast, excessive or high shearing led to a low methane production activity of D3 (0.24-0.26 L CH_4/g $COD_{removed}$) in period 1 and 2. In contrast to previous results by Jiang et al., [47] who reported a decrease in methane content in biogas due to shearing, in this study, biogas composition was not affected by the shearing. Indeed, all biogas samples were composed of approximately 60% methane and 40% carbon dioxide.

Following the experimental period 2, regeneration of D3 was conducted in period 3 and 4, respectively, by renewing 25% of the working volume each time (Table 6.1). The regeneration led to a notable recovery of biogas production (Figure 6.1) and methane production activity (Table 6.2), resulting in similar level of control system (digester D1) at the end of period 4.

These results reaffirm that excessive or high level of shearing could negatively affect the methanogenic activity.

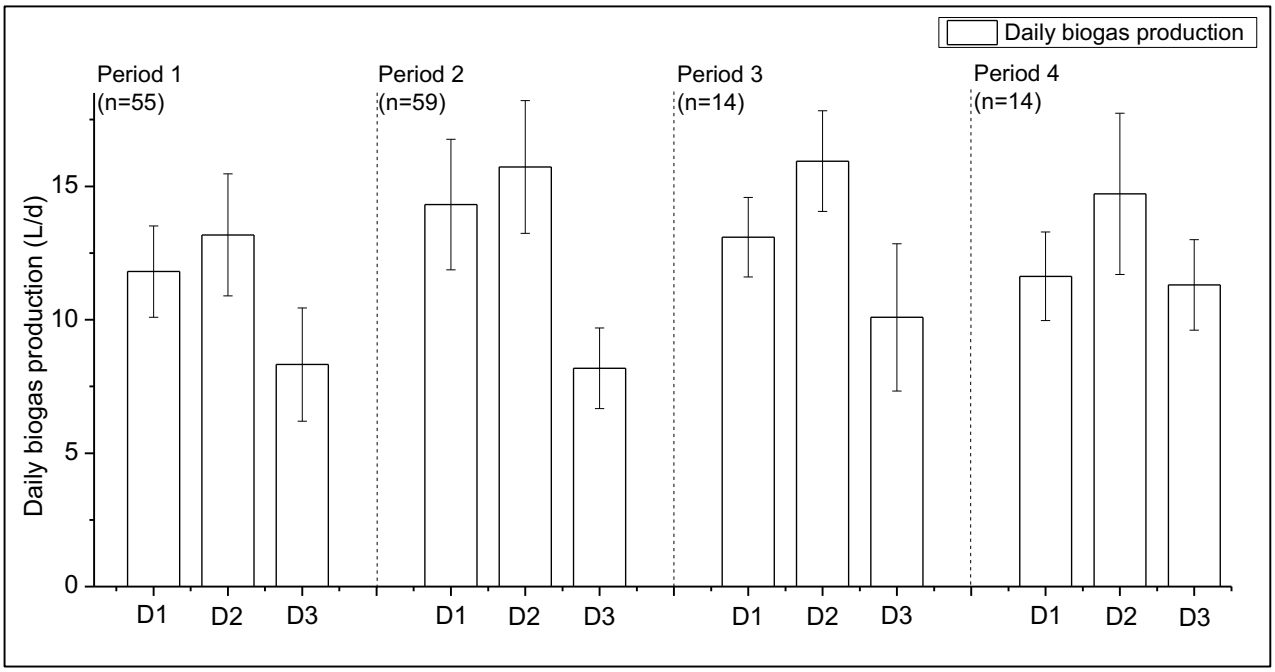


Figure 6.1 Daily biogas production from each individual digester. In the 3rd and 4th experimental period, the biomass in D3 was regenerated as described in section 2.1.3 while operation of D1 and 2 was the same as in period 2 (error bars show the standard deviation from eight measurements in period 1 and 2; and four measurements in period 3 and 4).

Table 6.2 Methane production activity and biogas composition during the experiment.

		D1	D2	D3
Period 1	Methane production acitivity (L CH_4/g COD $_{removed}$)	0.51	0.49	0.24
	CH_4/CO_2 (%/%)	60.4/38.1	59.8/38.5	58.1/39.0
Period 2	Methane production acitivity (L CH_4/g COD $_{removed}$)	0.49	0.52	0.26
	CH_4/CO_2 (%/%)	59.7/38.6	60.6/38.2	59.0/39.0
Period 3	Methane production acitivity (L CH_4/g COD $_{removed}$)	0.62	0.73	0.35
	CH_4/CO_2 (%/%)	59.6/39.5	59.6/37.9	60.2/39.1
Period 4	Methane production acitivity (L CH_4/g COD $_{removed}$)	0.49	0.66	0.56
	CH_4/CO_2 (%/%)	60.2/38.4	61.1/38.7	59.2/39.4

6.1.2 Sludge characteristics

Due to the temporal variation in TS and VS content of the primary sludge between wet and dry weather conditions, the removals of TS and VS by all three digesters were highly variable (Figure 6.2). There were similar variations in tCOD (from 19,000 – 39,000 mg/L) and sCOD (from 1,200 – 2,300 mg/L) in the primary sludge.

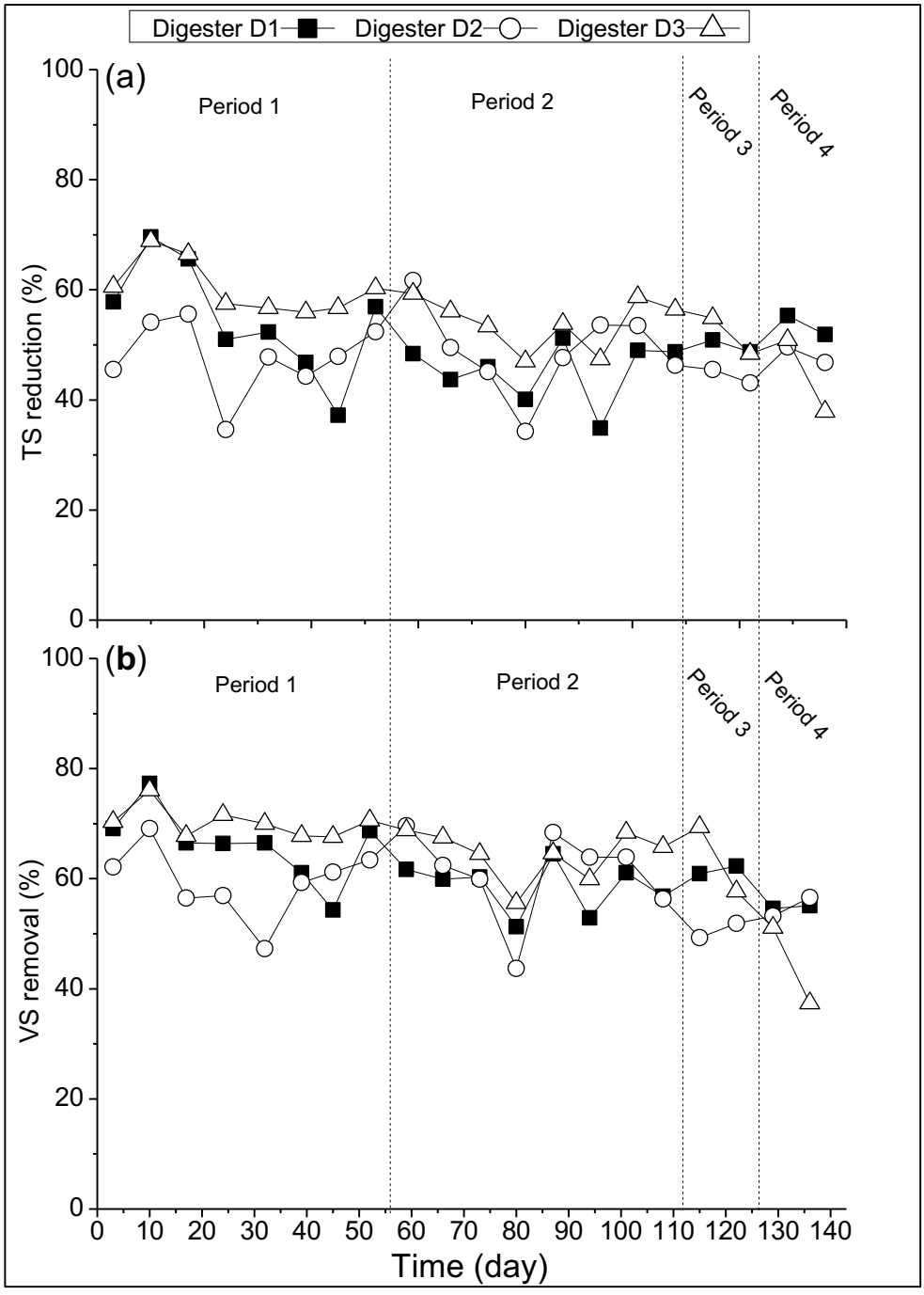


Figure 6.2 Removals of (a) TS and (b) VS by the three anaerobic digesters with recuperative thickening and different level of shearing.

Nevertheless, the effects of digestate shearing during recuperative thickening on both tCOD and sCOD removals by all three anaerobic digesters could be observed (Figure 6.3). Compared to the control digester (D1), digester D2 with medium shearing showed similar tCOD and sCOD removal efficiencies during the entire experimental periods (from 1 to 4). This observation is consistent with the methane production activity of D1 and D2 (Table 6.2). On the other hand, digester D3 with excessive and high shearing showed higher tCOD removal but lower sCOD removal during period 1 when excessive shearing was applied. In period 2, tCOD removal decreased to a similar of control digester (D1) when shearing was changed from excessive (using the food blender) to high (i.e. 600 rpm) as can be seen in Figure 6.3a. These results indicate that excessive shearing could solubilise some solid COD and the benefit from an increase in the soluble COD fraction in the substrate may offset any negative impact from cell rupture and exposure to oxygen during the recuperative thickening process. On the other hand, excessive or high level of shearing (digester D3) resulted in a significant increase in the sCOD fraction [49], thus, causing an increase in tCOD removal (Figure 6.3a) but a notable decrease in sCOD removal (Figure 6.3b).

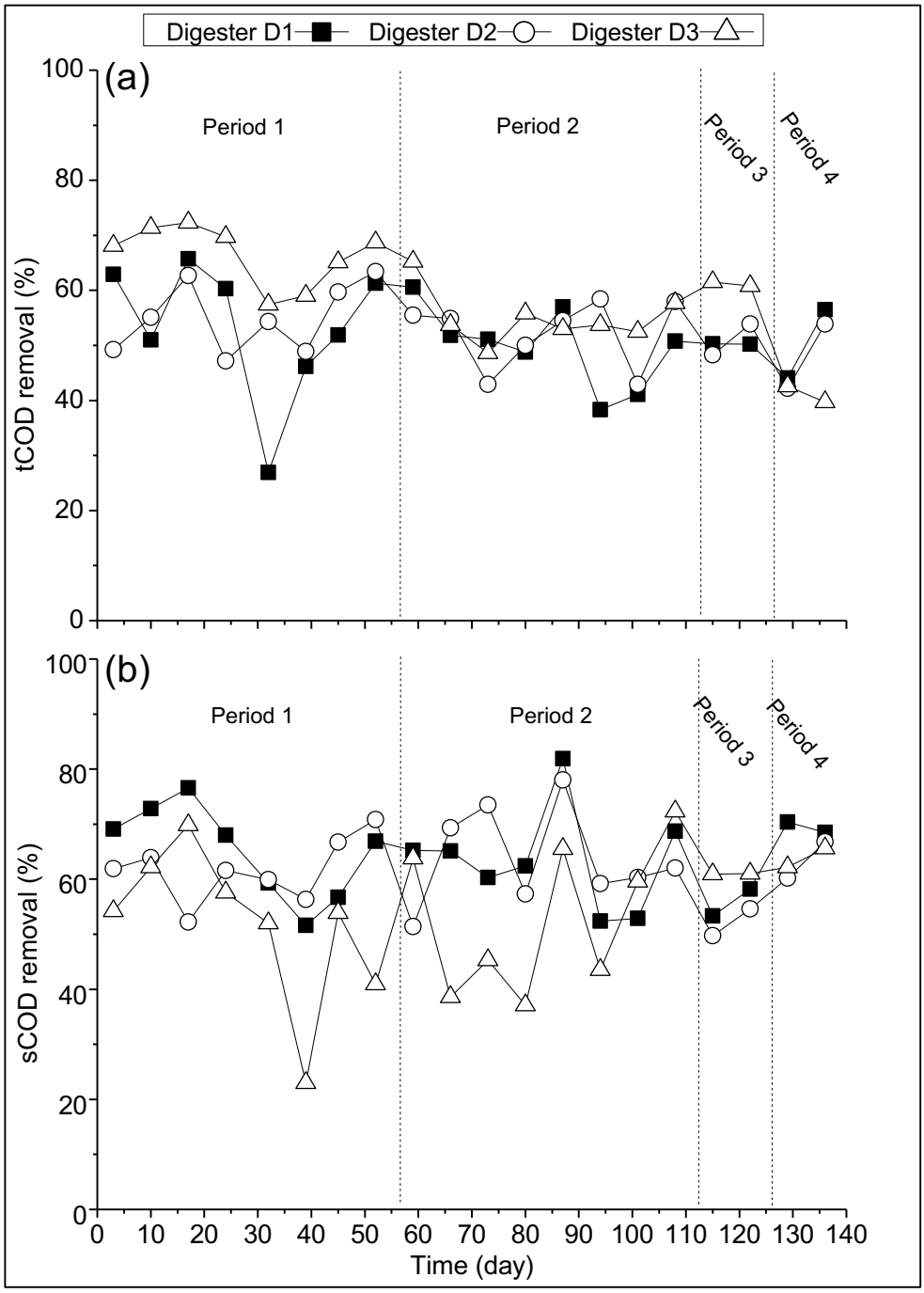


Figure 6.3 Removals of (a) tCOD and (b) sCOD by the three anaerobic digesters with recuperative thickening and different level of shearing.

It is noteworthy that the alkalinity at pH = 4.5 (Figure 6.4) and pH value of each digester were stable throughout the experiment. The mixed liquor pH of all three digesters was ranging from 7.01 to 7.72, which was typical for normal anaerobic digestion. Alkalinity of all digesters was also stable, ranging from 2700 to 3600 mg$CaCO_3$/L. Overall; all three digesters were in good condition throughout the current study. There was no indication of volatile fatty acid or ammonia accumulation in the digesters.

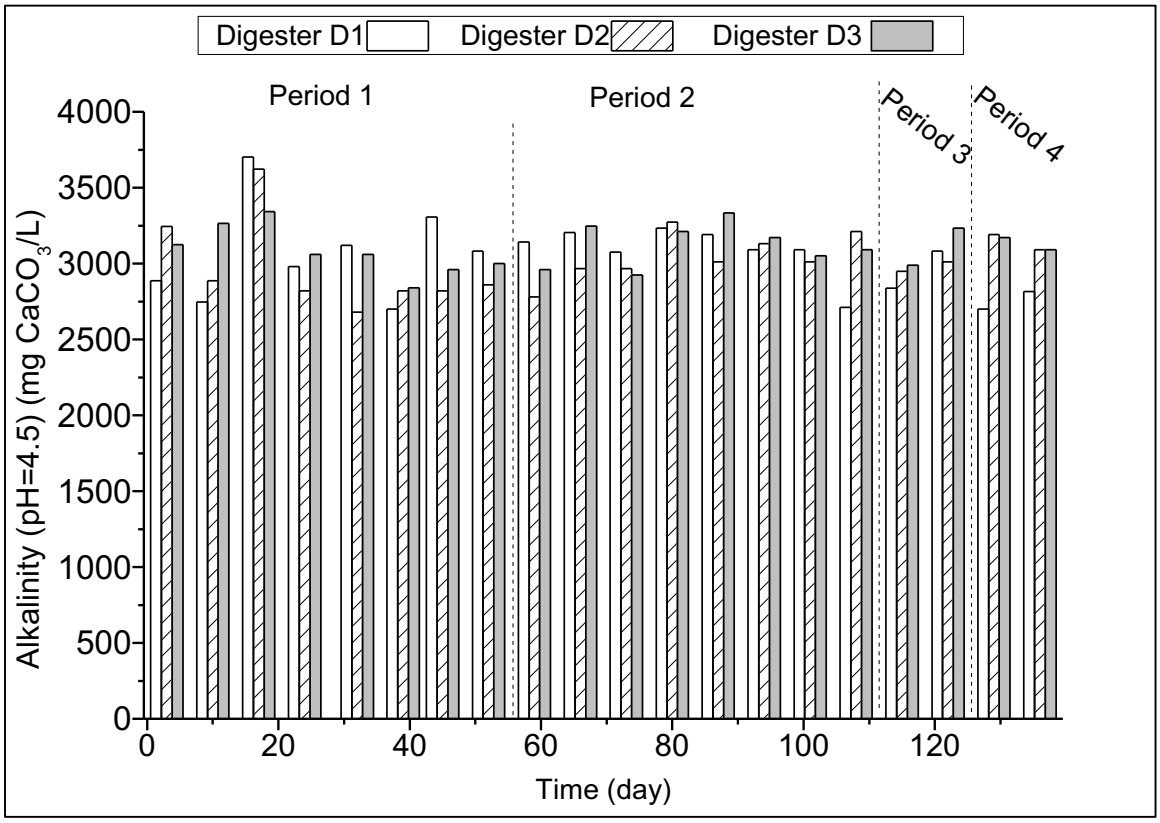


Figure 6.4 Alkalinity of each digester during the experiment.

6.1.3 tCOD balance of anaerobic digesters

In addition to the biogas production and COD removals during the experiments, the tCOD balance can also reveal the organic matter consumption and accumulation during the anaerobic processes (Figure 6.5).

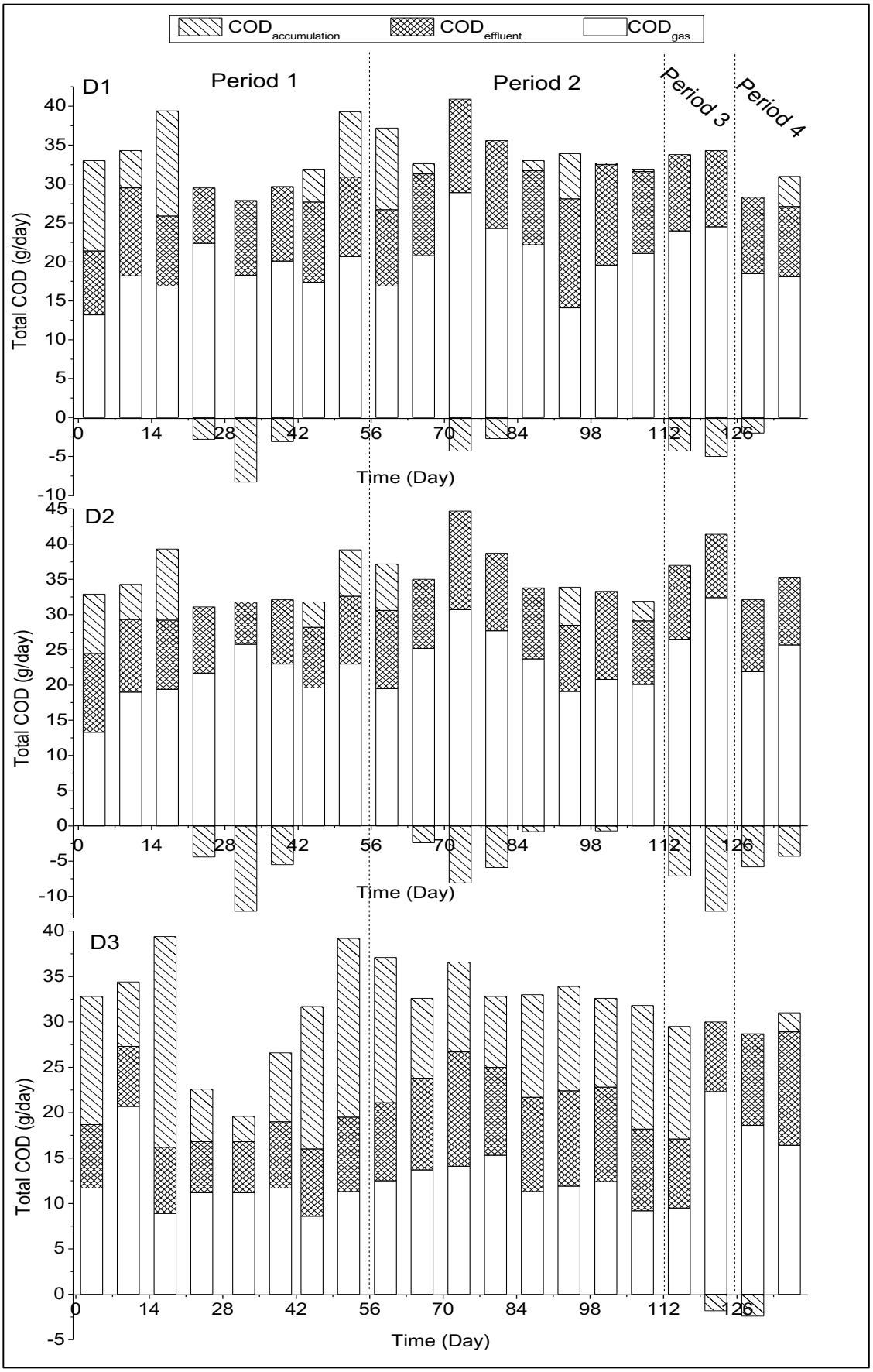


Figure 6.5 COD mass balance for 3 digesters during the experimental periods.

Compared to control digester D1, digester with medium shearing (D2) converted more tCOD into biogas during the experimental periods and left less organic matters in the digester. This result was corresponded to the better biogas production and methane production activity of D2 observed in Figure 6.1 and Table 6.2, respectively. However, under different shearing levels during period 1 and 2 (high shearing or excessive shearing), digester D3 had remarkably reduced organic matter which converted to biogas, leading the $COD_{accumulation}$ much higher than the other two digesters during the same period. It is expected that organic matter will be more accumulated in the digester when less biogas was produced under the same feeding condition. It can be confirmed by the observation of digester D3 in period 3 and 4, when biogas production was recovered to the similar level of control level, the accumulated COD of D3 was significantly reduced (Figure 6.5). Therefore, medium shearing would help to reduce the organic matter content in the digester and covert more organic matter to biogas, which would lower the cost for the following sludge treatment process like dewatering and disposal.

6.1.4 Sludge dewatering and odour compounds occurrence in the dewatered sludge

At the end of experimental period 1 and 2, sludge from each digester was collected to obtain dewatered sludge cake to elucidate the dewaterability and analysis the odour compounds of the sludge. Table 6.3 demonstrates the dry solid content of dewatered sludge cake from each digester. The dry solid content of sludge cake from digester D1 and D2 were similar during the same period, and fluctuating between 20-22.5% during experimental periods. However, dewatered sludge cake from digester D3 had remarkably reduced dry solid content (17.9%) when excessive shearing was applied. This observation should be correlated to excessive shearing applied to sludge during the thickening process. It has been reported by other studies that extracellular polymeric substances (EPS) was essential to sludge-floc formation, however, excessive EPS could weaken cell attachment and floc formation, leading to reduced dewaterability [236, 243, 244]. As shearing would lead to cell lysis and extract more EPS from the microbial cells, the dewaterability of D3 sludge would be reduced. Additionally, dewaterability of D3 sludge was increased slightly during experimental period 2, leading the dry solid content grows to approximately 19%. It is notable that reduced shearing to high level during period 2 did not recover the methanogenic activity (Figure 6.1 and Figure 6.2), and it had limited effect on the dewaterability as well.

Table 6.3 Dry solid content of dewatered sludge cake.

	Period 1		
	D1 (Control)	D2 (Medium shearing)	D3 (Excessive shearing)
Dry solid content (%)	21.3	22.5	17.9
	Period 2		
	D1 (Control)	D2 (Medium shearing)	D3 (High shearing)
Dry solid content (%)	20.2	21.9	19.4

Dewatered sludge cake was then collected in the sample bottles (60 g each bottle) to analysis the odour compounds according to the method mentioned in Section 3.4.3. Among all the odour compounds, hydrogen sulphide (H_2S) was the mostly detected volatile sulphide compound from the incubated sludge samples. The concentration of H_2S of each sludge sample (incubated for 8 days) was shown in Figure 6.6. Compared to control digester D1 and digester D3, dewatered sludge cake from digester D2 emitted least H_2S after 8 days' incubation in all experimental periods, while digester D1 and D3 had similar level of H_2S concentration. The reason could be due to the higher conversation of organic matter to biogas gas in digester D2 (Figure 6.5), leading to less organic sulphate compounds residue in the dewatered sludge cake from digester D2. Therefore, improved methanogenic activity caused by medium shearing was helpful to reduce the odour emission from sludge treatment and disposal process. It should be noted that the range of H_2S concentration in period 2 (270 – 320 μL/L) was much higher than period 1 (120 – 190 μL/L), which could be the reason of various feed sludge condition. As all digesters were fed with primary sludge collected from full-scale WWTP, the organic matter content in the feed would vary due to wastewater influent character and weather conditions etc.

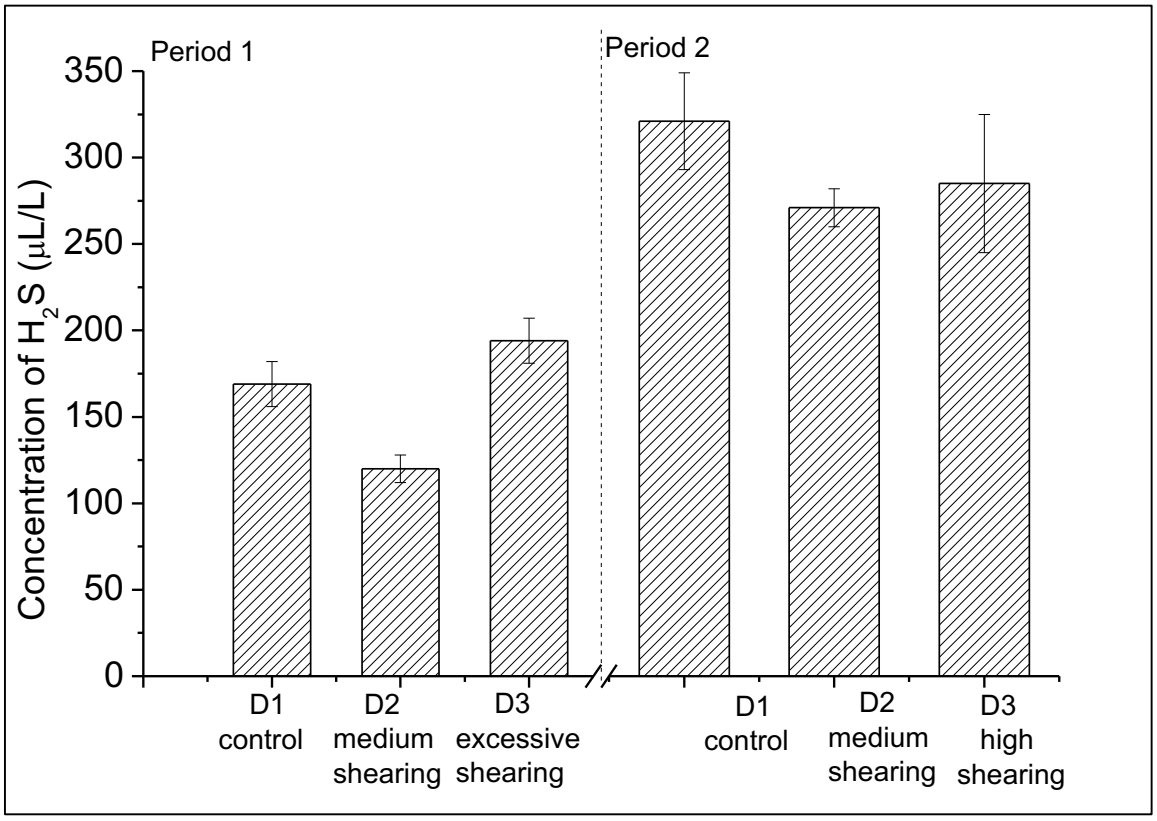


Figure 6.6 H_2S concentration of dewatered sludge cake from each digester. Average and standard deviation were taken from duplicate samples.

6.2 Impact of shear stress on microbial community dynamics

As describe in Chapter 3, Section 3.3.3, digested sludge samples from each digester were taken on the end of period 1 (day 55) and period 2 (day 110) to prepare DNA extraction samples and analysis of microbial community structure (Section 3.4.5). The microbial diversity, microbial community dynamics affected by the different shearing level will be discussed; additionally, the correlation of microbial community structure and digester performance will be also discussed.

6.2.1 Microbial diversity

Duplicated microbial community samples were taken at the end of period 1 (day 55) and 2 (day 110), respectively for each digester. Overall, 25 bacterial and one archaeal phyla were assigned for all samples and only very small number of sequences (1.7 ± 1.5%, n = 6) were not classified at this level (Figure 6.7). Major bacterial phyla were *Bacteroidetes* (31.9 ± 9.5%, n = 6), *Firmicutes* (17.5 ± 8.5%, n = 6), *Proteobacteria* (13.8 ± 3.6%, n = 6) and *Spirochaetes* (10.1 ± 9.7%, n = 6). Other bacterial phyla (*Acidobacteria, Actinobacteria, Caldiserica, Chloroflexi,*

Elusimicrobia, Fibrobacteres, OP8, Planctomycetes, SAR406, Synergistetes, Theromotogaes, Verrucomicrobia and *WWE1*) can present up to 10% of the sequences. The rare phyla (< 0.5%) were grouped into 'minor groups' (Figure 6.7) including *Chlorobi, Cyanobacteria, Fusobacteria, Lentisphaerae, NKB19, OP3, OP9,* and *WPS-2*. The sequence distribution among bacterial and archaeal phylogenetic groups in this study were well consistent with the core of microorganisms involved in anaerobic digestion systems [245].

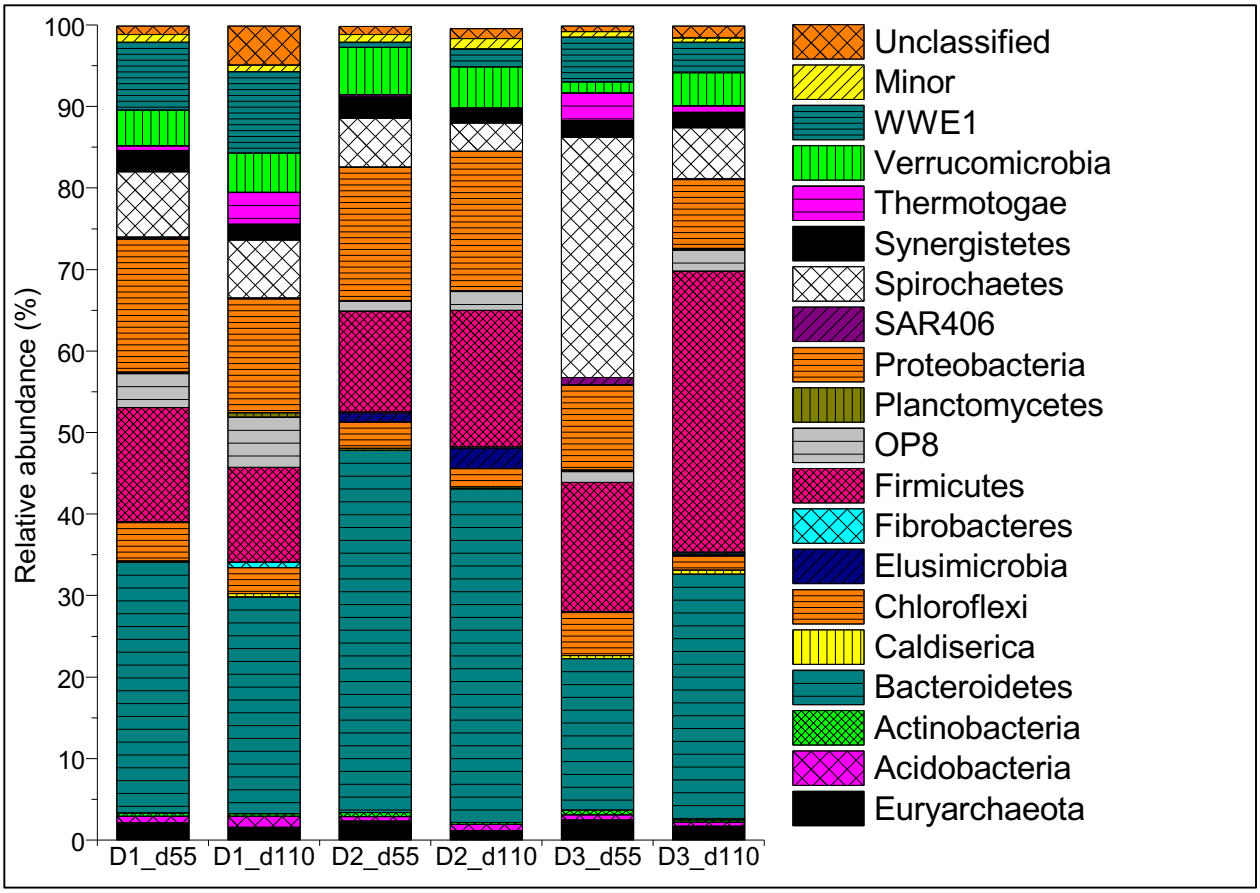


Figure 6.7 Relative abundance of microbial community at phylum level. Plotted values are mean of duplicate samples collected from three anaerobic digesters at day 55 and day 110 of experimental period: digester 1 (D1_d55 and D1_d110), digester 2 (D2_d55 and D2_d110) and digester 3 (D3_d55 and D3_d110). Microbial orders less than 0.5% in relative abundance were grouped in Minor.

The rarefaction curves (at 97% sequence similarity) from all samples were showed in Figure 6.8. Observed_species and phylogenetic diversity values showed the highest microbial diversity for digested sludge of D2 (medium shearing). Excessive shearing applied to D3 (sample D3_d55) led to the lowest microbial diversity, while reduced shearing level of D3 increased the microbial diversity (Observed_species and Phylogenetic diversity) at the end of period 2 (D3_d110). Based on Simpson index, sludge samples from D2 and D1 were more evenly distributed than those of D3. Similarly, Rochex et al., [246] reported a decrease of biofilm diversity under high shear stress (0.238 Pa) in biofilm formation system. The lower Simpson index of sample D3_d110 than that of sample D3_d55 probably indicated that the D3 may have not reached steady state after 55 d at high shearing level (600 rpm). Good_coverage showed more than 99% coverage for each sample (Table 6.4), indicating that only less than 1 additional OTU would be found if 100 additional sequences were provided.

*Table 6.4 Goods_coverage [(1-n/N)*100; where n and N are number of singletons and total number of sequences in sample, respectively] at even sequencing depth of 110000 sequences per sample (lowest sequencing noted among sample).*

Samples	D1_d55	D1_d110	D2_d55	D2_d110	D3_d55	D3_d110
Goods_coverage (%)	99.6 ± 0.0	99.7 ± 0.0	99.6 ± 0.0	99.6 ± 0.0	99.7 ± 0.0	99.6 ± 0.0

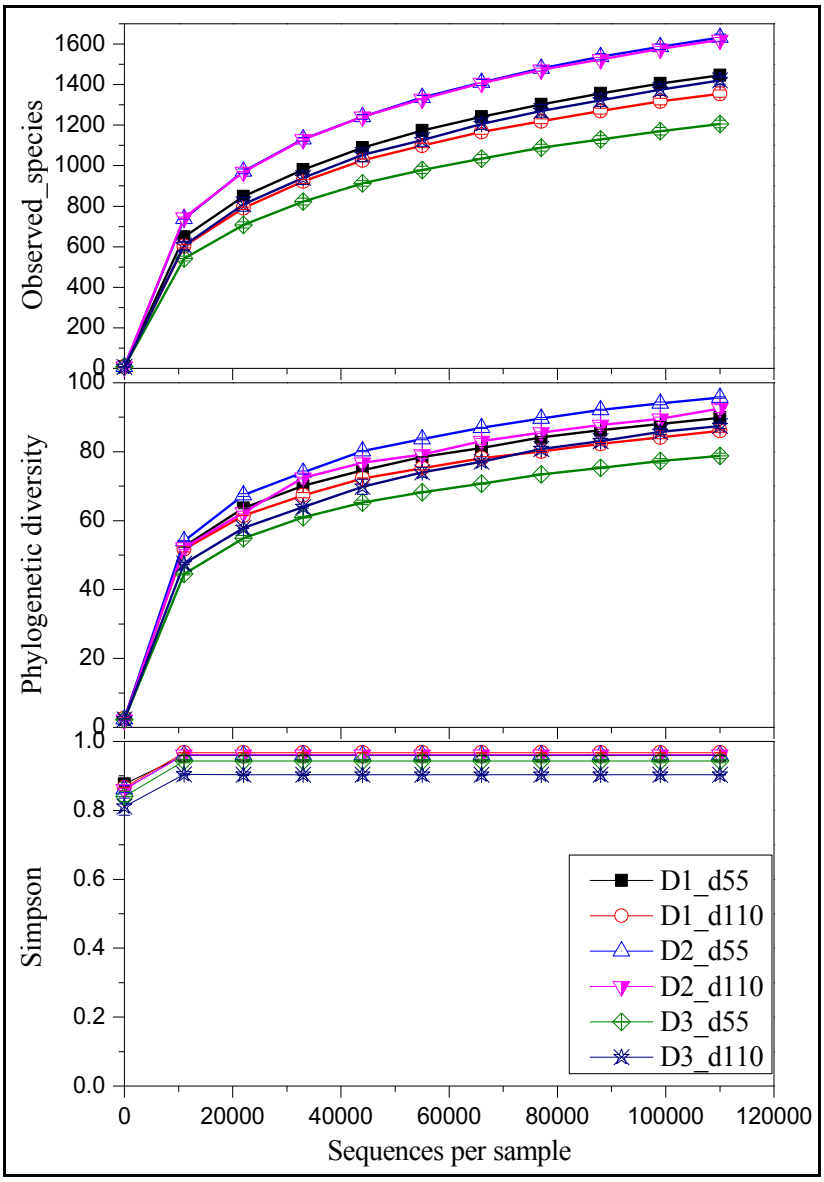


Figure 6.8 Rarefaction curves (at 97% sequence similarity) for Observed_species, Phylogenetic diversity and Simpson were analysed at event sequencing depth of 110000 sequences per sample (lowest sequence reads noted among samples). Error bars indicate standard deviation of duplicate samples collected from at day 55 and day 110 of experimental period for three anaerobic digesters: digester 1 (D1_d55 and D1_d110), digester 2 (D2_d55 and D2_d110) and digester 3(D3_d55 and D3_d110).

The weighted UniFrac distance metric, which based on the relative abundances of all phylotypes in a sample, was interpreted via PCoA (Figure 6.9). The close clustering within locations indicates that samples were more similar to each other in phylogenetic structure than they were to samples from other locations. As expected, all duplicate samples were plotted either very closely or overlapped with each other. Samples from D2 and D3 were clustered in

three groups along the PC1 vector (accounted for 59% variation) in corresponding to the applied shearing force (Figure 6.9): excessive shearing (D3_d55 of digester 3), high shearing (D3_d110 of D3) and medium shearing (all samples of D2). This result indicated a clear impact of applied shear force on microbial community structure. Effect of shear stress on the microbial community composition was previously reported for biofilm community [246] and anaerobic digestion population [49].

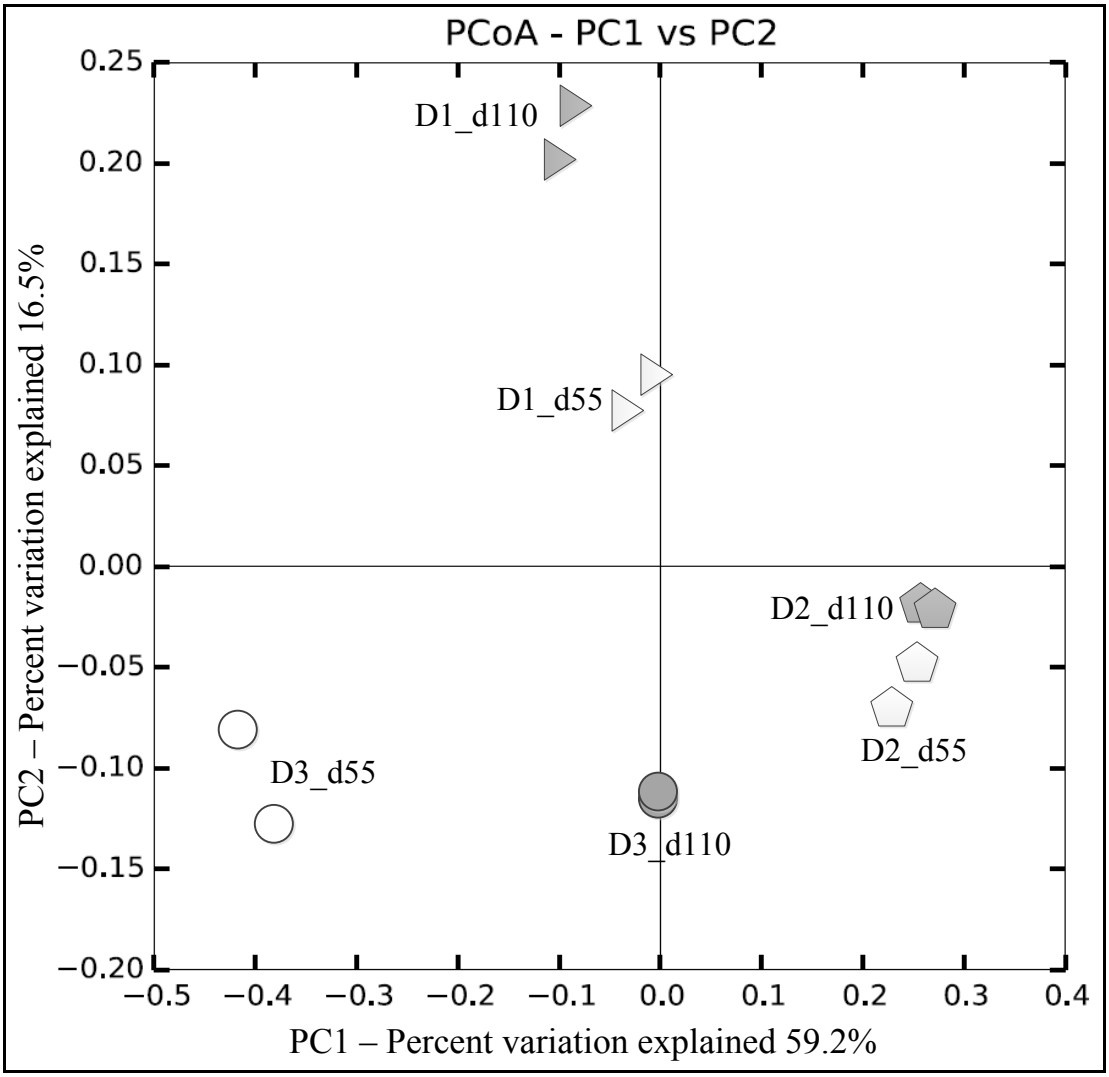


Figure 6.9 Phylogenetic distances between samples determined via weighted UniFrac principal coordinates analysis (distance matrix calculated at even sequencing depth of 110000 sequences per sample). Duplicate samples collected from three anaerobic digesters at day 55 and day 110 of experimental period: digester 1 (D1_d55 and D1_d110), digester 2 (D2_d55 and D2_d110) and Digester 3 (D3_d55 and D3_d110).

6.2.2 Dynamics of microbial communities

Taxonomic classification at order level was particularly focused to verify the dynamics of microbial communities. Overall, 50 microbial orders were identified and only small proportion (1.7 – 6.6%) of reads was unclassified at this level. Of which, 16 orders were accounted for more than 80% of the population abundance (Figure 6.10). *Bacteroidales* (31.6 ± 9.4%, n =6) was the most abundant order, following by *Clostridiales* (17.1 ± 8.6%, n = 6), *Spirochaetales* (8.7 ± 9.8%, n = 6), *Cloacamonales* (5.1 ± 3.6%, n =6) and *Syntrophobacterales* (5.0 ± 2.2%, n = 6). The most abundant archaeal population belonged to the order *Methanomicrobiales* (1.4 ± 0.4%, n =6).

In terms of relative abundance, a significant impact of shear stress was highlighted in four bacterial orders (*Bacteroidales*, *Clostridiales*, *Syntrophobacterales* and *Spirochaetales*) that were highly presented and known for important function in anaerobic digestion. *Bacteroidales* was the most abundance in D2 (medium shearing) (42.3 ± 2.3%, n =2), following by D1 (control) (28.4 ± 2.9%, n =2). This order was lowest in D3 when excessive shearing applied (18.4%), but it was significantly increased to 30% when switching to high level shearing for 55 days during period 2. The distribution of *Clostridiales* was quite stable in D1 and D2 (11.2 – 15.6%). However, their abundance in D3 was increased significantly from 15.6% to 34.4% when shearing was decreased from excessive to high level. *Bacteroidales* and *Clostridiales* are well known for their role in hydrolysis and fermentation [247-250]. Werner et al. [16] proposed that these bacterial groups relied more on redundancy to maintain the overall community function. The abundance of syntrophic division *Syntrophobacterales* was highest in D2 (from 5.8% in day 55 to 8.0% in day 110), following by D1 (from 5.3% in day 55 to 6.0% in day 110) and then lowest in D3 (from 2.1% in day 55 to 2.7% in day 110). *Syntrophobacterales* was a specialized group for metabolic function of short-chain fatty acid oxidation [251, 252]. *Syntrophobacterales* population was found to be the most sensitive to perturbation during anaerobic digestion processes. Due to its essential role, this bacterial group was observed to rebound after perturbation rather than replacing by other groups of similar function [16]. In contradictory to above three orders, *Spirochetales* (mainly genus *Treponema*, Figure 6.12) was particularly the most abundant order (28.5%) in D3 when excessive shearing applied, and it was significantly decreased to 5.2% when shearing level reduced in D3 for 55 days. The presence of *Spirochaetales* in D1 and D2 was low and slightly decreased from 6.8 % and 4.2% (day 55) to 5.0% and 2.3% (day 110), respectively (Figure 6.11). The function of *Treponema*

in anaerobic digestion was poorly understood. It may play a role on acetate production at the acetogenesis step [17] or relate to utilization of glucose [252].

Dynamic changes in bacterial community were also observed for other orders including *Burkholderiales, Rhodocyclales* (belonging to *β-Proteobacteria*) and *Synergistales*. These bacterial orders was reported to involve in utilization of fatty acids (propionate, butyrate or acetate) [252]. Overall, the trend of microbial communities observed in D1 and D2 showed the increase of even distribution of the bacterial phylotypes from day 55 to day 110, the decrease of abundant phylotypes as well as increase of minor groups (Figure 6.11). A greater evenness of community was considered as an indicator of better performance of anaerobic digestion process [16].

Archaeal population was present at low abundance in all samples with only one phylum *Euryarchaeota* (1.2 – 2.5%). No significant variation between samples was observed for this population (Figure 6.11). The most abundant order was *Methanomicrobiales* (0.8 – 2%), following by *Methanosarcinales* (0.1 – 0.35%), E2 (belonging to *Thermoplasmata*, < 0.4%) and *Methanobacteriales* (< 0.2%). Syntrophic association between Clostridiales (mainly genus Clostridium, Figure 6.10) populations and hydrogenotrophic methanogens (Methanomicrobiales) has been reported in the literature [247, 248]. Such syntrophic association can explain for the prevalence of Methanomicrobiales compared to other archaea as observed here. It is noted that the primer pairs 341F/806R applied in this experiment was not specialized to target archaeal, so it probably led to underestimate the archaeal population. However, Hanreich et al., [253] observed that methanogenic population represented less than 4% of the community, but protein of archaeal origin accounted for 20 – 30% of the identified protein, suggesting a disproportional active of methanogens.

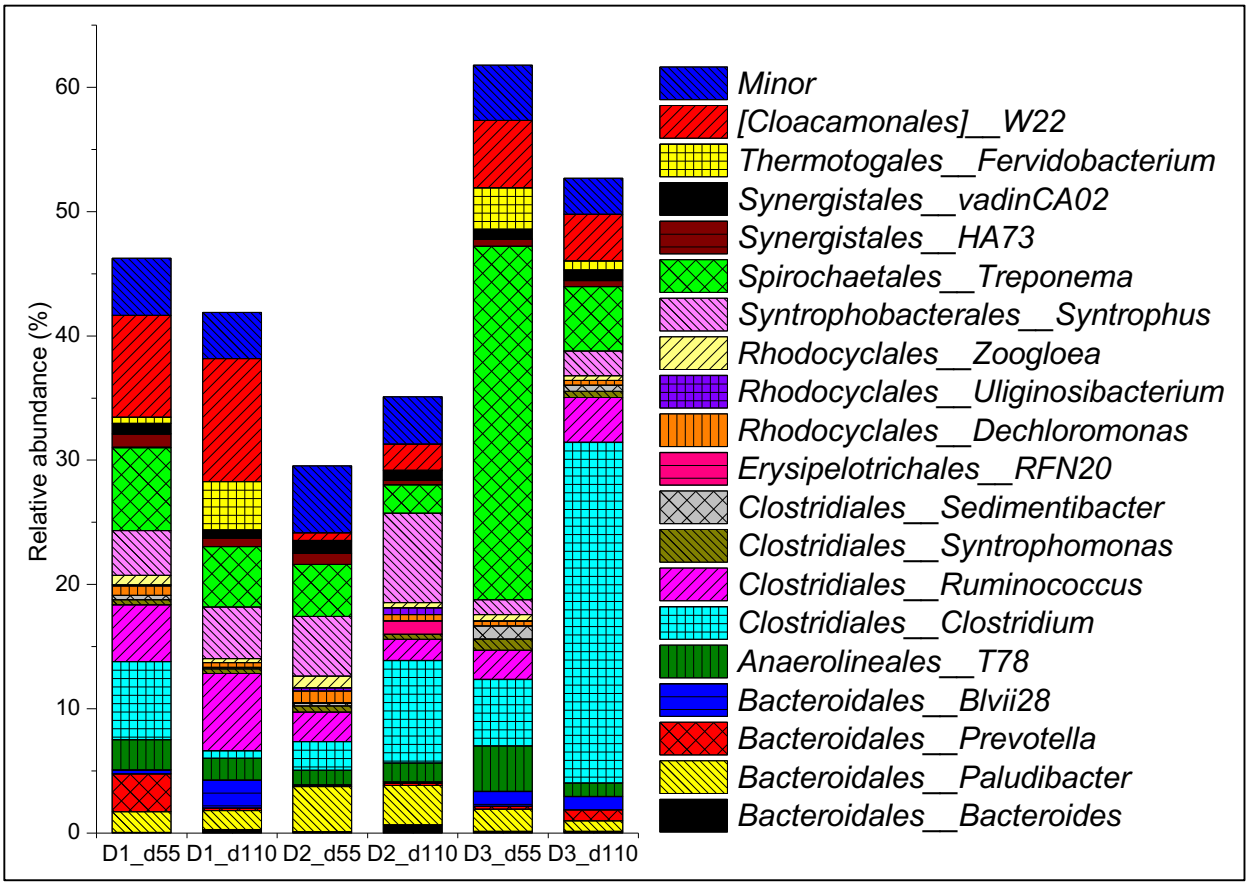


Figure 6.10: The most abundant genera (relative abundance >0.5% in at least one of the samples). Plotted values are mean of duplicate samples collected from three anaerobic digesters at day 55 and day 110of experimental period: digester 1 (D1_d55 and D1_d110), digester 2 (D2_d55 and D2_d110) and digester 3 (D3_d55 and D3_d110). Microbial genera less than 0.5% in relative abundance were grouped in Minor. Unclassfied sequences ranged from 35% (D3_d55) to 66% (D2_d55), and were not plotted in the graph to have a better visualization.

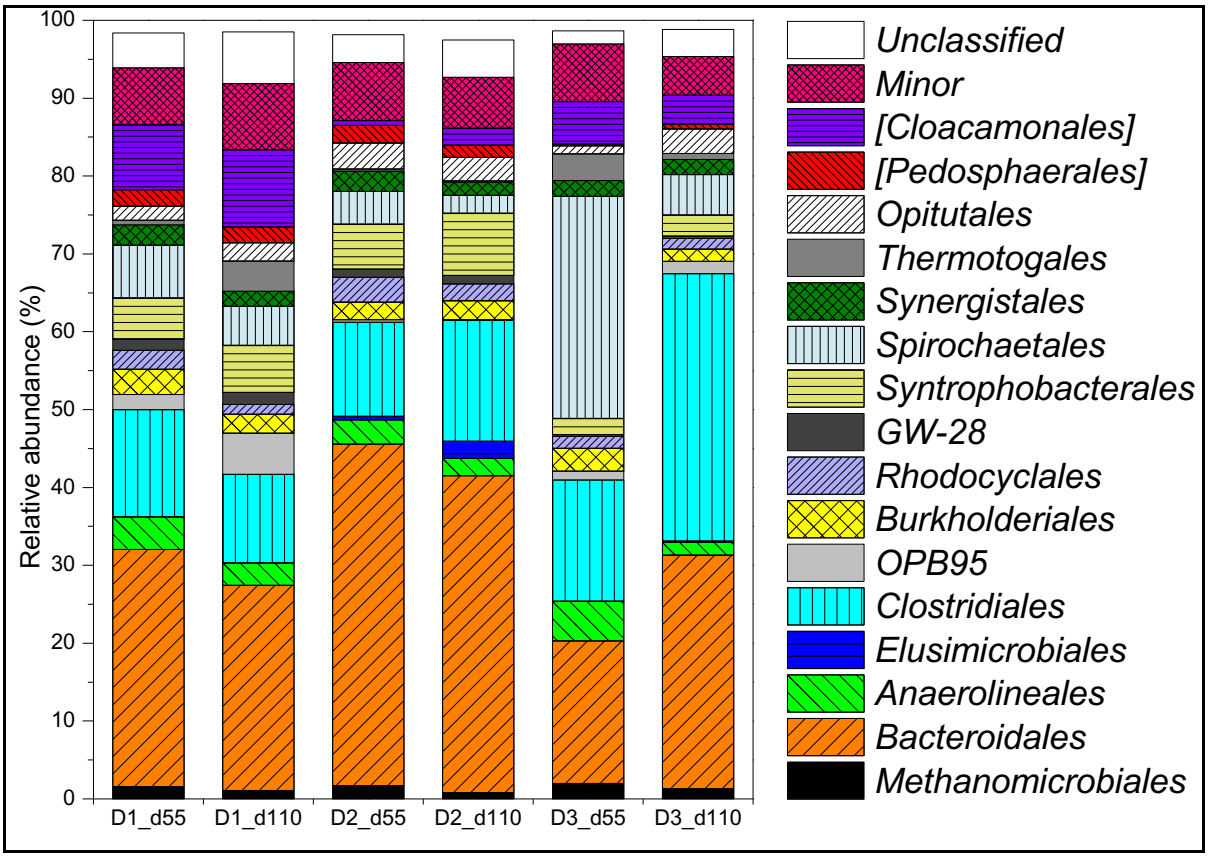


Figure 6.11 Relative abundance of microbial community at order level. Plotted values are mean of duplicate samples collected from three anaerobic digesters at day 55 and day 110 of experimental period: digester 1 (D1_d55 and D1_d110), digester 2 (D2_d55 and D2_d110) and digester 3 (D3_d55 and D3_d110). Microbial orders less than 1.5% in relative abundance were grouped in Minor. The sum did not reach 100% since operational taxonomic units (OTUs) less than 0.05% was filtered from OTU table.

6.2.3 Correlation between digester performance and microbial community structure

A good correlation between microbial diversity and reactor performance was observed in this study. D2 with medium shearing sustained the development of microbial communities with higher diversity and evenness (Figure 6.8) that was well correlated with a better biogas production (Figure 6.1). These results highlighted the importance of microbial diversity and evenness of anaerobic digestion communities. In addition, these results are also consistent with previous findings that microbial community diversity, evenness of microbial community structure and microbial community dynamics over time are important ecological parameters to maintain functional stability and robustness of anaerobic digesters [254]. Anaerobic digestion

communities with greater evenness and phylogenetic variability could function more efficiently [16]. Taxonomic classification demonstrated the dynamic of microbial community over time. It also indicated the impact of shear force on important functional bacterial groups. The abundance and stable of *Bacteroidales* and *Clostridiales*, important hydrolytic and fermentative bacteria, in digester D2 resulted in higher capacity to use redundant functional pathways to maintain the efficiency of the system. The resilient abundance of *Syntrophobacterales* increased over time, particularly in digester D2, which emphasized on their specialized function in short-chain fatty acid oxidation [15]. It is also indicated that excessive or high level of shearing in digester D3 did not favour the *Bacteroidales* and *Syntrophobaterales*, which worked as hydrolyzer and acetogens, respectively, in the anaerobic digestion process, and led to reduced biogas production for digester D3 [15]. Despite the lack of specific Archaeal target primers, the syntrophic association between *Clostridiales* (mainly genus *Clostridium*) populations and hydrogenotrophic methanogens (*Methanomicrobiales*) was demonstrated. Excessive shearing created the condition that highly favoured the development of *Spirochaetales* (mainly *Treponema*). Probably, the high available sCOD/organic matters released during excessive shearing process in digester D3 explained for this high abundant of *Treponema*.

6.3 TrOC occurrence and their removals during different shearing levels

In addition to the digester performance and microbial community structure, TrOC occurrence in primary sludge and TrOC removals by anaerobic digestion with different shearing level were also studied. Sludge samples were taken from feed (primary sludge) and digesters weekly, and prepare the TrOC samples following the method mention in Chapter 3, Section 3.4.4.

6.3.1 Occurrence of TrOC in the primary sludge

Of the 40 TrOCs monitored here, 17 compounds were consistently detected in all primary sludge samples in either the liquid or solid phase (Figure 6.12). They include 12 pharmaceuticals and personal care products, 2 pesticides, 2 industrial chemicals (i.e. TCEP and bisphenol A), and one stimulant (i.e. caffeine). Their occurrence in primary sludge is not a surprise given their wide spread use both at household level and in the industry. However, the concentrations of these TrOCs, in either aqueous or solid phase, varied significantly (Figure 6.12). Several compounds were observed with high average concentration in aqueous phase

(>10,000 ng/L) and elevated concentration in the solid phase (1340 to 7940 ng/g dry sludge). These include caffeine, paracetamol, triclosan and triclocarban. The prevalent occurrence of these TrOCs in the wastewater sludge is attributed to their prevalent use in our modern society. For examples, caffeine is a stimulant in tea, coffee, and some energy drinks. Paracetamol is an over-the-counter analgesic and antipyretic drug. Triclosan and triclocarban are antibacterial/antifungal agents widely used in soap, detergent, and toothpaste. In addition, several TrOCs such as ibuprofen and bisphenol A were also occasionally detected at high concentrations in both the aqueous and solid phase. In addition, some lipophilic TrOCs may partition from the aqueous to the solid phase of sewage [59], resulting in significantly high concentrations (several μg/kg dry weight or more) in sludge. It is noted that the high standard deviation shown in Figure 6.12 also indicates a significant temporal variation in their occurrence in primary sludge. Primary sludge samples were taken from a full-scale wastewater treatment plant; thus, weather conditions and other temporal variations can influence the concentration of TrOCs.

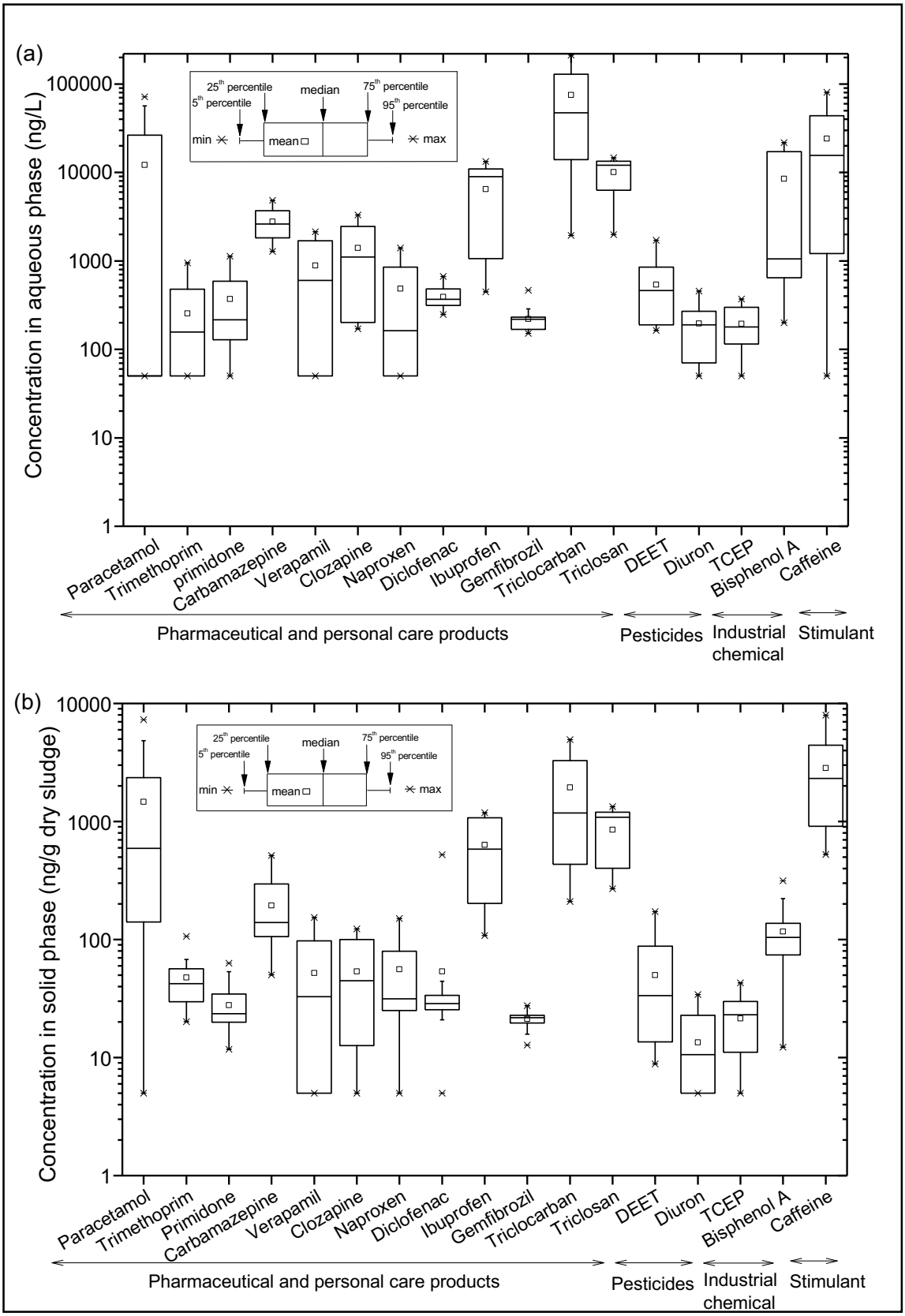


Figure 6.12 TrOC concentrations in (a) aqueous phase and (b) solid phase of primary sludge. 22 samples were taken during the three months' experimental period.

6.3.2 TrOC removal from the aqueous phase

Given the interaction between the aqueous phase and solid as well as the partition of TrOCs between these two phases, it is essential to examine their fate in each phase separately and to ascertain their overall removal efficiency. Among the 17 TrOCs detected in the primary sludge, the removal of highly hydrophilic (logD < 1) and readily biodegradable TrOCs from the aqueous phase was not significantly affected by shearing conditions. These TrOCs include caffeine, trimethoprim, paracetamol and naproxen which were reported to be well removed by anaerobic digestion in the literature [58, 255-257] due to the presence of electron donating functional groups in their molecular structure, rendering them susceptible to nucleophile attack (i.e. biodegradation). Indeed, these TrOCs were well removed by all three digesters (control, medium shearing and excessive/high shearing) with trimethoprim under excessive shearing conditions being an exception (Figure 6.13).

In contrast to the highly hydrophilic TrOCs, all other TrOCs detected in this study showed negligible removal from the aqueous phase regardless of the shearing levels. These poorly removed TrOCs can be classified as moderately to highly hydrophobic given that their logD values ranged from 1 to 6 at pH of 7. The results in Figure 6.13 highlight the distinction between anaerobic digestion of sewage sludge and anaerobic treatment of wastewater. The former is fed with primary sludge with high solid content (e.g., 24.5±2.1 g/L in this study) while the feed solution (i.e. wastewater) to the latter contains very little solid content. Indeed, several hydrophobic TrOCs such as triclosan and triclocarban have been reported to be well removed from aqueous phase [215, 226] during anaerobic membrane bioreactor treatment. In this study, release of some hydrophobic TrOCs including bisphenol A, triclosan and triclocarban from the solid phase to the aqueous phase was manifested by a higher concentration in the aqueous phase after anaerobic digestion compared to that in the primary sludge. This was due to the change in pH from 5.3-5.6 in the primary sludge to 7.1 - 7.5 in the digester (digested sludge), leading to an increased solubility of these TrOCs.

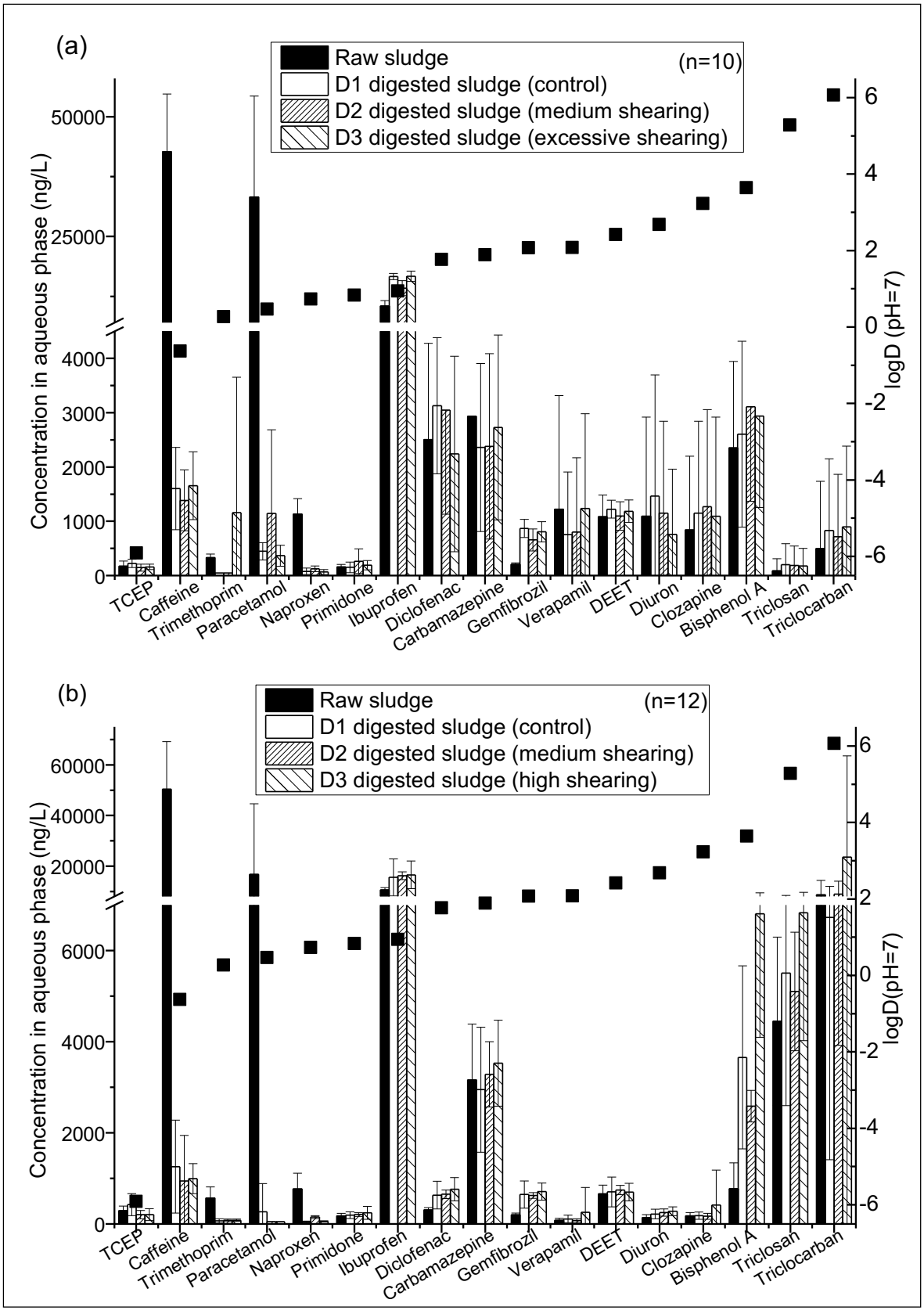


Figure 6.13 TrOCs concentrations from the aqueous phase of raw primary and digested sludge of each digester during (a) Stage 1 and (b) Stage 2. Error bars represent the standard deviation of 10 and 12 measurements in Figure 6.13a and 6.13b, respectively.

6.3.3 TrOC removal from solid phase

As discussed above, anaerobic digestion of sewage sludge is a solid dominated system. In other words, the absolute mass of TrOCs in the solid phase is expected to be higher than that in the aqueous phase. Thus, TrOC concentration in the solid phase is expressed as ng/g rather than ng/kg dry sludge Figure 6.14. It is also noteworthy that recuperative anaerobic digestion achieved approximately 60% TS reduction. Thus, since data presented in Figure 6.14 show the TrOC concentration in the solid phase of primary sludge and digested sludge of each digester, caution is required when examining TrOC removal from the solid phase using Figure 6.14. For instance, approximately 60% removal of triclosan can be inferred from Figure 6.14 although the concentration of triclosan in the primary sludge was the same as in the digested sludge. A more systematic approach to quantify TrOC removal in discussed in the next section based on an overall mass balance.

Figure 6.14 clearly shows that hydrophilic and readily biodegradable TrOCs, such as caffeine, trimethoprim, and paracetamol, were also well removed from solid phase regardless of the shearing condition. Noting the reduction in the TS content, an increase in TrOC concentration in the solid phase can be observed for all moderately to highly hydrophobic TrOCs. For a few TrOCs (e.g. carbamazepine, verapamil, clozapine, and triclocarban), the increase in solid phase concentration was significant, indicating no or very low biodegradaion in the solid phase. This observation is consistent with results previously reported in the literature and is due to their hydrophobicity and the presence of electron withdrawing functional groups (such as chloro and amide) in their molecular structure [256-258]. However, it is noteworthy that previous studies have not attempted to evaluate the overall removal of TrOCs through a systematic mass balance (which will be discussed in the next section).

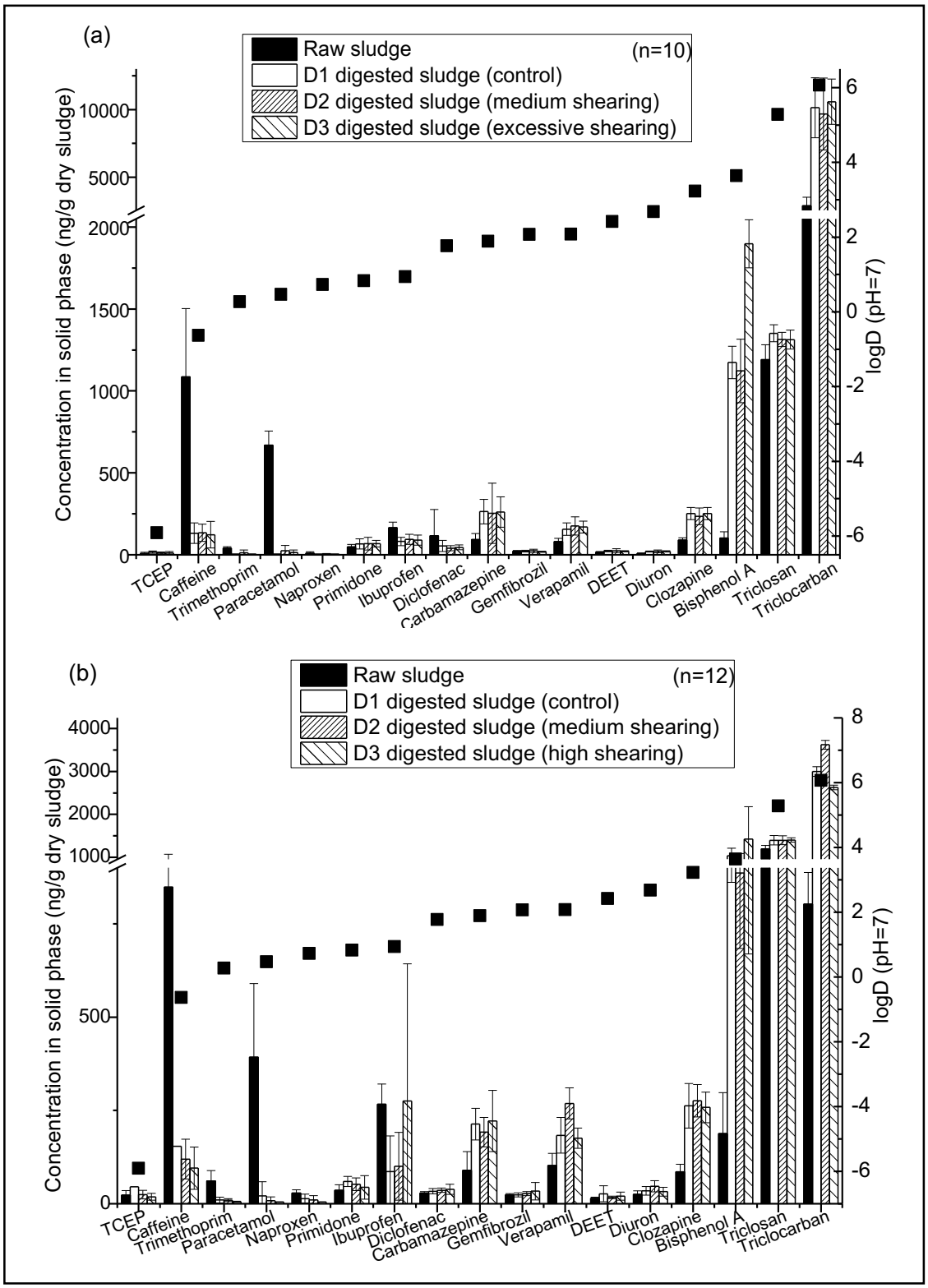


Figure 6.14 TrOCs concentrations from the solid phase of raw primary and digested sludge of each digester during (a) Stage 1 and (b) Stage 2. Error bars represent the standard deviation of 10 and 12 measurements in Figure 6.14a and 6.14b, respectively.

6.3.4 The mass distribution of TrOCs during the sheared anaerobic digestion

A mass balance was conducted based on Equations 3-6 – 3-8 (section 3.4.4, Chapter 3) to systematically examine the interplay between biodegradation and the partitioning of TrOCs between the aqueous and solid phase (Figure 6.15). This figure shows a wide range of overall removal (via biodegradation) of the 17 TrOCs ubiquitously detected in raw primary sludge in this study. Under the control (no shearing) and medium shearing conditions, recuperative anaerobic digestion resulted in over 90% removal (via biotransformation) of caffeine, trimethoprim, paracetamol, and naproxen. These compounds are among the most hydrophilic TrOCs (Figure 6.15a and b). They also have electron donating functional groups (e.g. hydroxyl) in their molecular structure, which are known to make the compound more biodegradable [215, 228]. TCEP is an industrial chemical occurring in an ionic form in the aqueous phase, and thus, is highly hydrophilic. However, the molecular structure of TCEP contains three carboxylic groups which are known to have strong electron withdrawing activity. Thus, TCEP removal via biodegradation by recuperative anaerobic digestion is not significant. In fact, all TrOCs containing electron withdrawing functional groups in their molecular structure only exhibited low to moderate removal. Several compounds with strong electron withdrawing functional groups (Table 6.5) including ibuprofen, diclofenac, carbamazepine, gemfibrozil, DEET, diuron, and triclocarban did not show any discernible removal by recuperative anaerobic digestion. These results are consistent with that in previous studies in which TrOC removal by anerobic treatment was investigated [215, 255]. Several other studies have also reported the positive influence of electron donating functional groups on the biodegradation of the TrOCs under aerobic treatment conditions [228, 259].

The impact of shearing on the removal of several TrOCs could be observed in Figure 6.15. Medium and excessive shearing resulted in a notable increase in the removal (i.e. biodegradation) of TCEP, diclofenac, DEET, and triclosan (Figure 6.15). It is possible that medium and excessive shearing facilitate the circulation of these relatively persistent TrOCs between the solid and aqueous phase, making them more available for biodegradation. However, these results were not observed for the remaining TrOCs investigated in this study. In fact, there is a notable decrease in trimethoprim removal (via degradation) under excessive shearing compared to medium and no shearing conditions (Figure 6.15). This may be explainable by the deteriorating biological performance of the digester at excessive shearing (section 6.1); however, given the low concentration of trimethoprim in both the aqueous

(255±250 ng/L) and solid (48±23 ng/g) phase in the primary sludge (Figure 6.12), experimental error cannot be completely ruled out. To further examine the above hypothesis that shearing could promote the circulation of TrOCs between the aqueous and solid phase, thus, influencing their biodegradation during anaerobic digestion, mass distributions of TrOCs in the digester during high and excessive shearing conditions were compared in Figure 6.16.

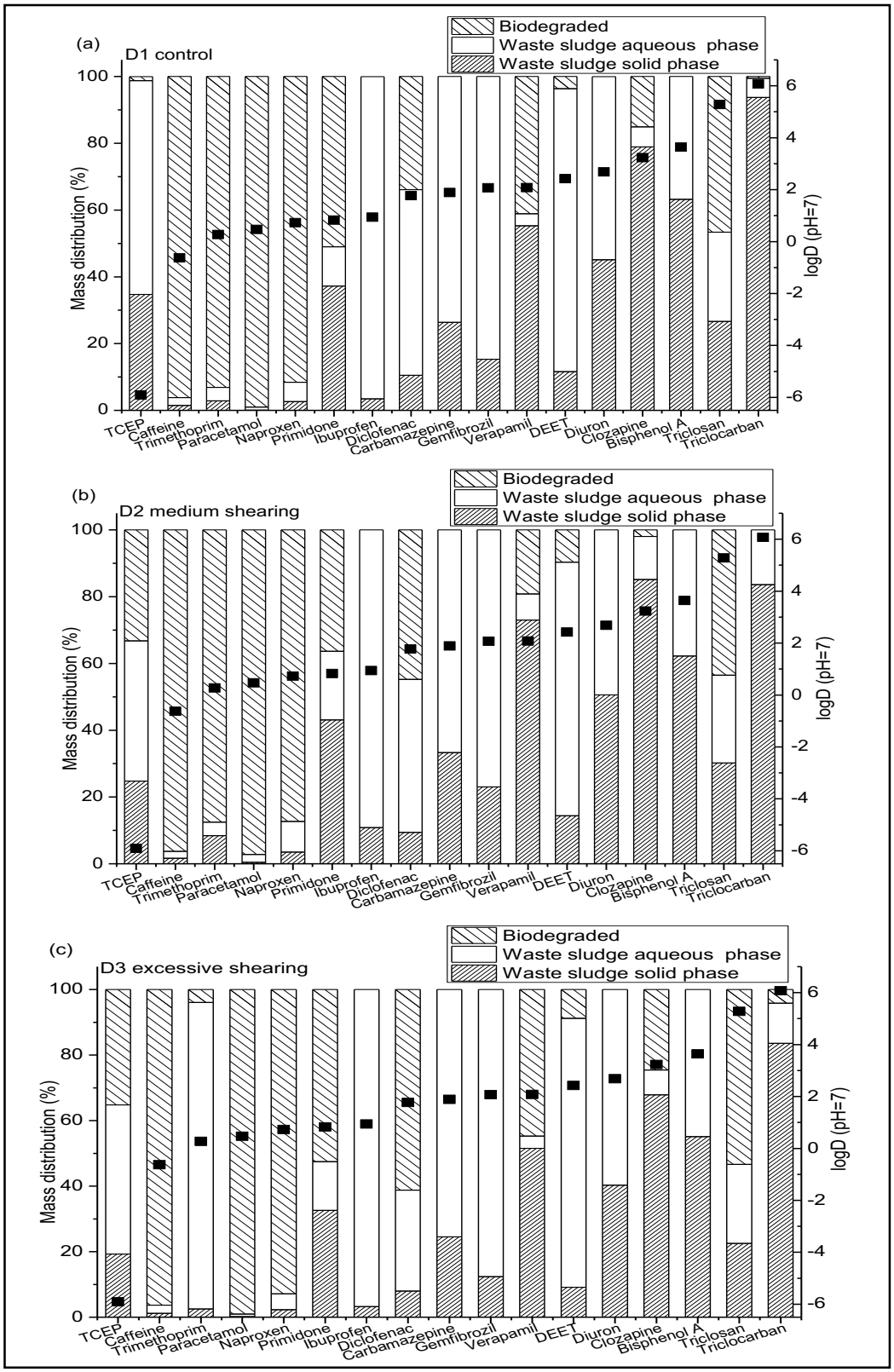


Figure 6.15 TrOCs mass distribution in anaerobically digested sludge with (a) no shearing (D1); (b) medium shearing (D2); and (c) excessive shearing (D3) were applied during experimental stage 1.

Results reported in Figure 6.16 further highlight the impact of shearing on the biodegradation of TrOCs by recuperative anaerobic digestion. Under a high shearing condition, some biodegradation of all 17 TrOCs detected in the primary sludge was observed. These include TrOCs that previously showed no biodegradation under no or medium shearing conditions. It is still evidenced that TrOCs with strong electron withdrawing functional groups in their molecular structure are less biodegradable (lower removal) than the others in Figure 6b. These results confirm that the circulation of TrOCs between the aqueous and solid phase could facilitate their biodegradation. Furthermore, results in Figure 6.16 also suggest excessive shearing is counterproductive as it significantly compromises the biological activity of the digester.

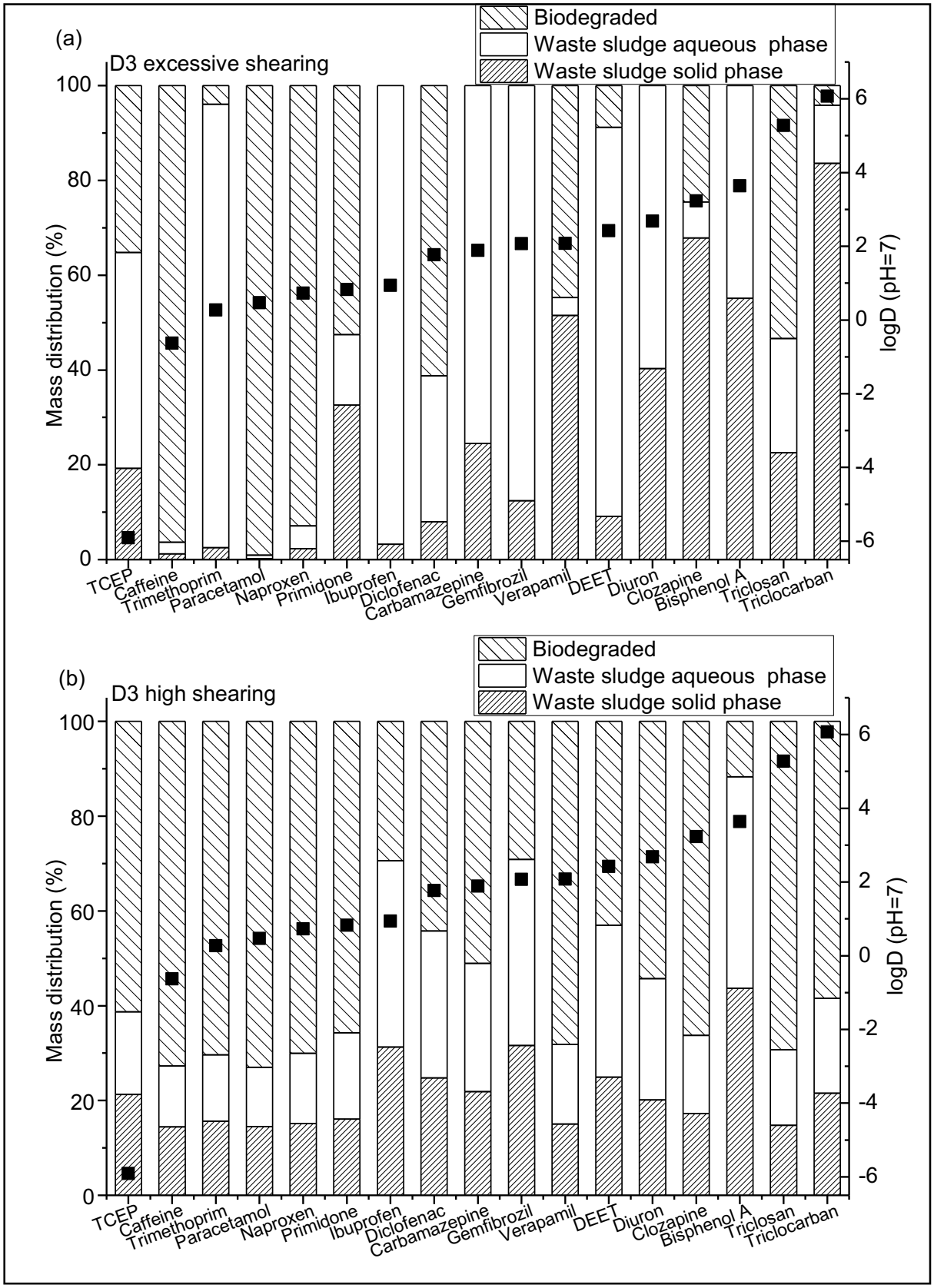
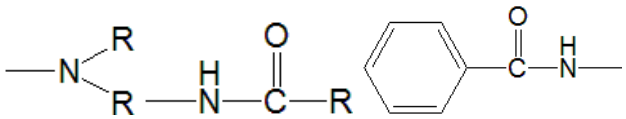
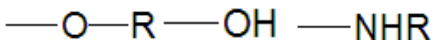
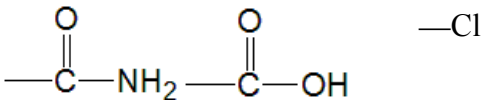
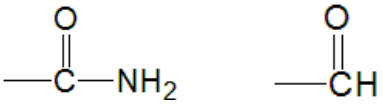


Figure 6.16 TrOCs mass distribution in digested sludge from D3 during (a) excessive shearing and (b) high shearing conditions.

Table 6.5 Electro donating and withdrawing functional groups found in TrOCs detected in this study.

Strong electron donating functional group	Strong electron withdrawing functional group
 	 

It is interesting to note that 15 TrOCs were constantly detected in the study in Chapter 4 and this chapter. The following Table 6.6 compares these TrOCs' biodegradation under the experimental conditions.

A few compounds like caffeine, paracetamol, naproxen, were well biodegraded under all conditions, indicating that they are highly biodegradable regardless the SRT or shearing levels. These results are also in accordance with previous discussion that strong electro donating functional groups benefit the biodegradation of compounds. On the other hand, compounds with strong electron withdrawing groups, like carbamazepine, gemfibrozil, diuron and triclocarban, were resistant under most conditions. It is notable that increment of SRT did not affected the biodegradation of most compounds, which was consistent of Chapter 4' findings that TrOCs were not the major substrates for microbial activity of anaerobic digestion process. However, decreased microbial activity deteriorated the biodegradation of selected compounds such as ibuprofen and triclosan, while improved microbial activity under medium shearing improved the biodegradation of some persistent compounds like carbamazepine and diuron.

Table 6.6 Biodegradation of selected TrOCs under different experimental conditions

Experimental conditions	HRT=20 d SRT=20 d	HRT=20 d SRT=30 d	HRT=20 d SRT=30 d medium shearing	HRT=20 d SRT=30 d high shearing	HRT=20 d SRT=30 d excessive shearing
Caffeine	96%	96%	96%	72%	96%
Trimethoprim	81%	93%	88%	70%	4%
Paracetamol	99%	99%	97%	72%	99%

Experimental conditions	HRT=20 d SRT=20 d	HRT=20 d SRT=30 d	HRT=20 d SRT=30 d medium shearing	HRT=20 d SRT=30 d high shearing	HRT=20 d SRT=30 d excessive shearing
Naproxen	93%	91%	87%	70%	93%
Primidone	0%	51%	36%	65%	53%
Ibuprofen	21%	0%	0%	30%	0%
Diclofenac	4%	33%	45%	44%	61%
Carbamazepine	0%	0%	0%	51%	0%
Gemfibrozil	0%	0%	0%	29%	0%
Verapamil	49%	41%	19%	68%	45%
Diuron	0%	0%	0%	54%	0%
Clozapine	41%	15%	2%	66%	26%
Bisphenol A	54%	0%	0%	12%	0%
Triclosan	54%	46%	44%	69%	53%
Triclocarban	0%	1%	0%	58%	4%

6.4 Conclusions

In this study, different levels of shearing force were applied during the thickening process for the anaerobic digestion with reparative thickening. Three digesters with different shearing levels were operated in parallel. An agitator at 300 rpm and 600 rpm was used for suppling medium and high level shearing, respectively during the thickening process, and a food blender was used to apply excessive shearing. The results showed that digester D2 with medium shearing during the thickening process produced most biogas/methane; while excessive or high levels of shearing deteriorate the methane yield significantly during the experiment. Biogas composition was not affected by the shearing. Indeed, all biogas samples were composed of approximately 60% methane and 40% carbon dioxide. TS and VS removals were not affected by shearing force. But excessive or shearing was observed to improve the tCOD removal and lower the sCOD removal during the experiment. The reason could be that shearing could solubilise some solid COD and the benefit from an increase in the soluble COD fraction in the

substrate may offset any negative impact from cell rupture and exposure to oxygen during the recuperative thickening process.

Shearing force was also reported to influence the microbial community structure of digestate. It was observed that medium shearing improved the diversity and evenness of microbial community which led to improved digestion performance, whilst excessive shearing was not beneficial to hydrolyzer and acetogens of anaerobic digestion, leading to deteriorative digestion performance.

The prevalent occurrence of 17 TrOCs in sewage sludge was demonstrated. Hydrophilic and readily-biodegradable TrOCs were well removed regardless of shearing conditions. On the other hand, shearing can facilitate the circulation of TrOCs between the aqueous and solid phase, thus, enhancing the biodegradation of some TrOCs. Under high shearing conditions, some biodegradation of all 17 TrOCs prevalently occurred in primary sludge (including those that showed no biodegradation under no or medium shearing) was observed.

Chapter 7 Enhancing the performance of anaerobic digestion by thermal pre-treatment and recuperative thickening

Anaerobic digester is a group of biological processes which consists of hydrolysis, fermentation, acetogenesis and methanogensis, among which hydrolysis of organic matter is the first and the rate limiting step of anaerobic digestion [260]. Therefore, various preatreatment methods, including thermal pre-treatment, biological treatment, ultrasonic and ozone, have been suggested to increase the digestion rate or improve the inherent degradability of the complex material [92, 94, 95, 112, 114, 261]. Thermal pretreatement is an efficient pre-treatment method to improve methane production during anaerobic process, due to the breakdown of organic waste into short-chain fragments that are better suited for biological digestion by microorganisms [31, 99]. As reported in previous studies, various temperature ranging from 120 – 180 °C and treatment duration up to 2 hours have been tested to indicate the effect of thermal pre-treatment on anaerobic digestion [32, 100, 101, 103-105, 108, 109]. Thermal hydrolysis helps to increase biogas/methane production of anaerobic digestion, and it also results in increased hydrolysis rates for both batch tests and pilot digesters. Bougrier et al. [104] reported that the optimal temperature of thermal hydrolysis is 150-180 °C and treatment duration is 30-60 mins. In addition to thermal pre-treatment, Chapter 5 has elucidated that recuperative thickening is also recognised as method to improve anaerobic digester performance. Recuperative thickening can increase sludge retention time (SRT) from hydraulic retention time (HRT) by removing water from a proportion of digestate and returning thickened sludge to digester [38, 262, 263]. In the previous studies, recuperative thickening increases biogas production, sCOD removal etc.

Apart from the anaerobic digestion performance, trace organic contaminants (TrOCs) were also another interest in this project. Chapter 4 and Chapter 6 have revealed the TrOCs removal and fate during anaerobic digestion when different SRTs and shearing levels were applied, respectively. Some researchers also reported the varying impact of thermal pre-treatment on TrOC removal from sludge. Thermal hydrolysis of primary sludge at 150 °C for 2 hours was found deteriorating the biodegradation of nonylphenol in a lab-scale mesophilic anaerobic digester [264]. Carballa et al. [265] found that thermal pre-treatment of mixed sludge at 130 °C

for 1 hour had no impact on the removal of various pharmaceuticals, musks, and hormones. Therefore, it is of great importance to reveal the TrOCs fate during anaerobic digestion when thermal pre-treatment was occurred to feed of anaerobic digestion.

This chapter aims to evaluate the influence of thermal pre-treatment and recuperative thickening on the anaerobic digester performance; furthermore, the TrOCs concentrations from sludge samples were analysed to reveal the occurrence of TrOCs and their fate during anaerobic digestion.

Three digesters were operated in parallel; the detailed regime is shown in Table 7.1. In brief, digester D1 was a control digester with SRT of 20 d and feeding with original primary sludge. Digester D2 and D3 were fed with pretreated primary sludge. In addition, digester D3 was operated with recuperative thickening to achieve an SRT of 30 d with the HRT at 20 d i.e., same as the other digesters. A thickening ratio of 1.33 (which is the ratio of the total TS from primary sludge feed and return thickened sludge over the TS from primary sludge feed) was used. A pressure vessel was used to provide thermal pretreament to the primary sludge at 150 °C for 30 mins, and the treated sludge was cooled down to room temperature before feeding. Sludge samples were collected to analysis sludge character parameters and TrOCs concentrations every week.

Table 7.1 Anaerobic digester operation regime with pre-treatment and recuperative thickening.

Digester	D1	D2	D3
HRT (d)	20	20	20
SRT (d)	20	20	30
Feed primary sludge volume (L/d)	1	1	1
Thermal pre-treatment (150 °C, 30 mins)	No	Yes	Yes
Recuperative thickening	No	No	Yes (thicken ratio = 1.33)

7.1 Anaerobic digesters performance improvement by thermal pre-treatment and recuperative thickening

7.1.1 Biogas production and sludge characters

Thermal pre-treatment and recuperative thickening (Digester D3) resulted in approximately 15% increase in biogas production in comparison to the control digester (D1) (Figure 7.1) The combination of thermal pre-treatment and recuperative thickening (Digester D3) did not lead to any additional increase in biogas production compared to only thermal pre-treatment (D2). According to Pilli et al. [266], thermal pre-treatment can induce the disintegration and solubilisation of solid sludge particles, thus, enhancing the hydrolysis step and hence biogas production. Indeed, in this study, in which approximately 10% of the tCOD of primary sludge was converted to sCOD after thermal treatment. On the other hand, recuperative thickening can extend the residence time of sludge in the reactor and recapture soluble macro-organic molecules for further digestion. Biogas production increase by up to 30% has been reported in previous laboratory scale and full scale studies. Results from Figure 7.1 suggest that the benefits of thermal pre-treatment and recuperative thickening are mutually exclusive. It is also noteworthy that thermal pre-treatment and recuperative thickening did not exert any observable impact on biogas composition. Throughout this study, biogas composition from all three digesters was stable with approximately 60% CH_4 and 40% CO_2.

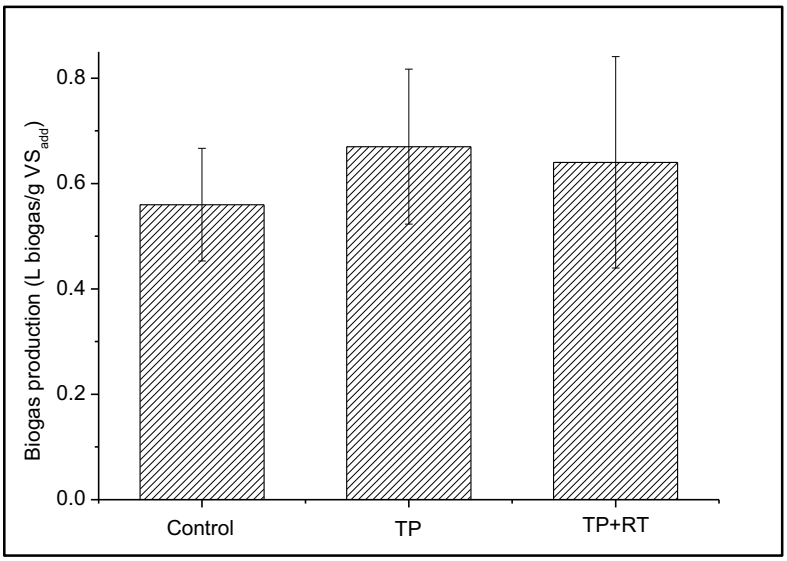


Figure 7.1 Average biogas production from digester D1 (Control), D2 (Thermal pre-treatment (TP)) and D3 (Thermal pre-treatment and recuperative thickening (TP+RT)). Error bars show the standard deviation of 7 measurements (one per week).

The sludge composition varied quite significantly throughout the course of this study. Since organic removal in terms of TS, VS, tCOD and sCOD determined on a weekly basis, there was some notable variation. TS and VS removals were ranging from 50 to 80% and 70 to 90% respectively (Figure 7.2). Due to these significant variations in TS and VS, the effects of thermal pre-treatment and recuperative thickening were not observable in this study. Nevertheless, some enhancement in tCOD removal could be observed in Figure 7.3a. With the exception of day 49, the removal of tCOD by Digester 2 (thermal pre-treatment) and Digester 3 (thermal pretreament and recuperative thickening) was comparable or higher than that of the control digester (D1) Figure 7.3a).

The effect of pre-treatment and recuperative thickening on removal performance was most observable in terms of sCOD removals. Digester D2 showed comparable sCOD removal to that by the control digester (D1). On the other hand, digester D3 showed a notable increase in sCOD removal (Figure 7.3b). As noted above, thermal pre-treatment led to the solubilisation of some tCOD into sCOD. On the other hand, due to sludge thickening, soluble organics can be retained for further digestion. Thus, recuperative thickening could improve the removal of sCOD.

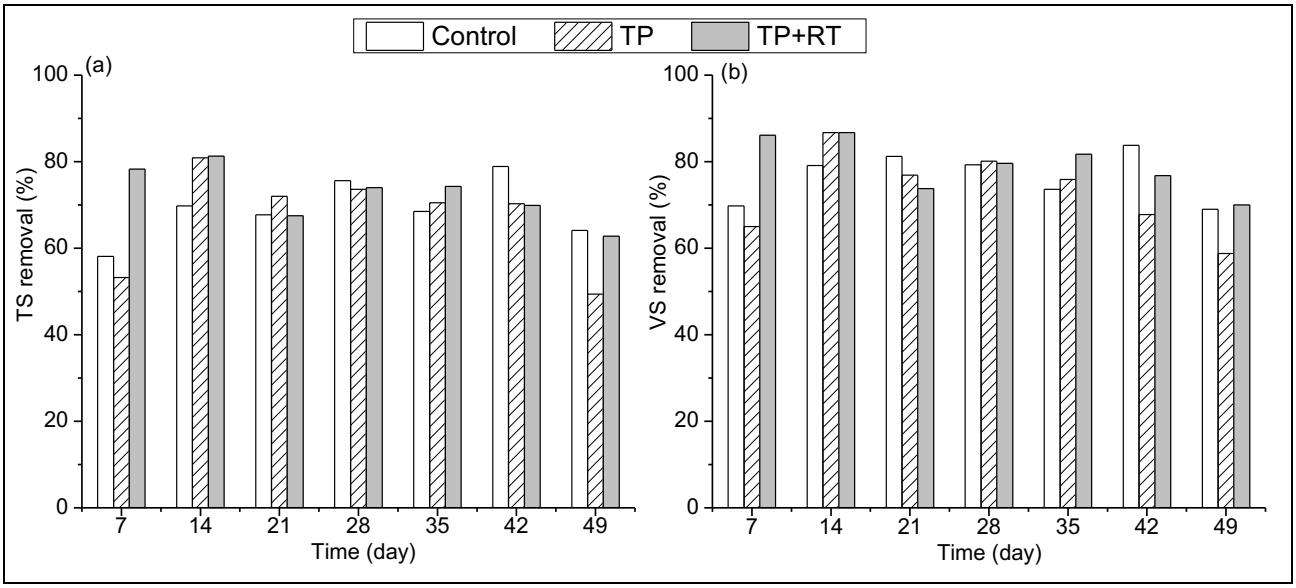


Figure 7.2 (a) TS removal and (b) VS removal by digester 1 (control), digester 2 with thermal pre-treatment (TP), and digester 3 with thermal pre-treatment and recuperative thickening (TP+RT).

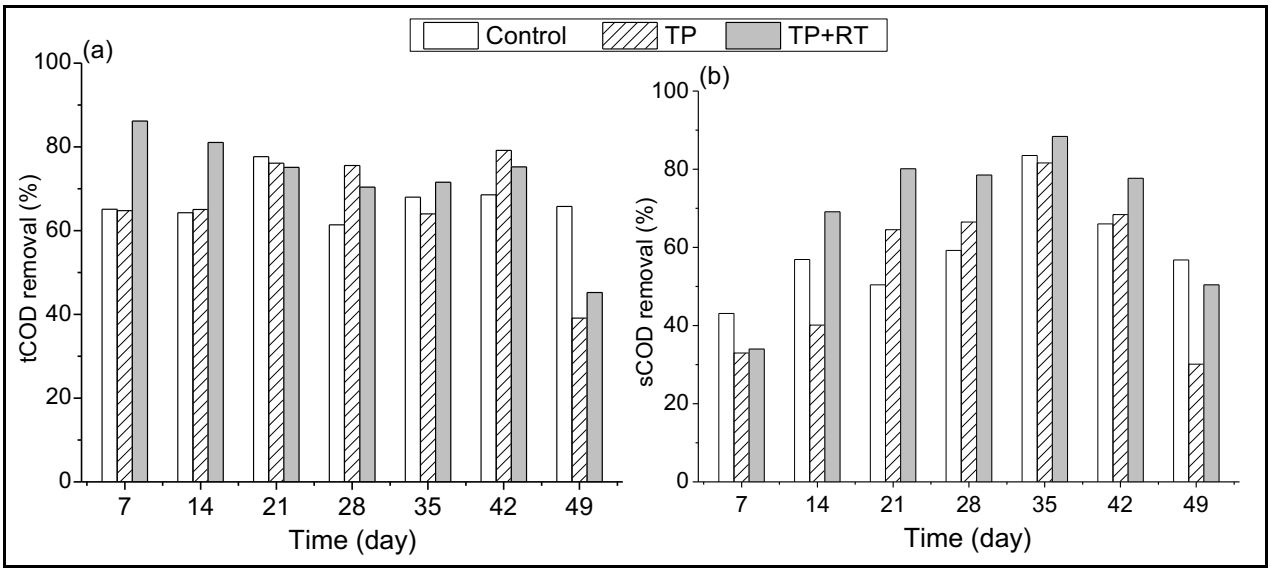


Figure 7.3 (a) tCOD removal and (b) sCOD removal by the digester 1 (control), digester 2 with thermal pre-treatment (TP), and digester 3 with thermal pre-treatment and recuperative thickening (TP+RT).

Several other parameters including pH and alkalinity were also monitored. The mixed liquor pH value of all three digesters was stable between 7.0 – 7.5 and the alkalinity was over 2600 mg $CaCO_3$/L (Figure 7.4). These results confirm stable operation of all three digesters in this study.

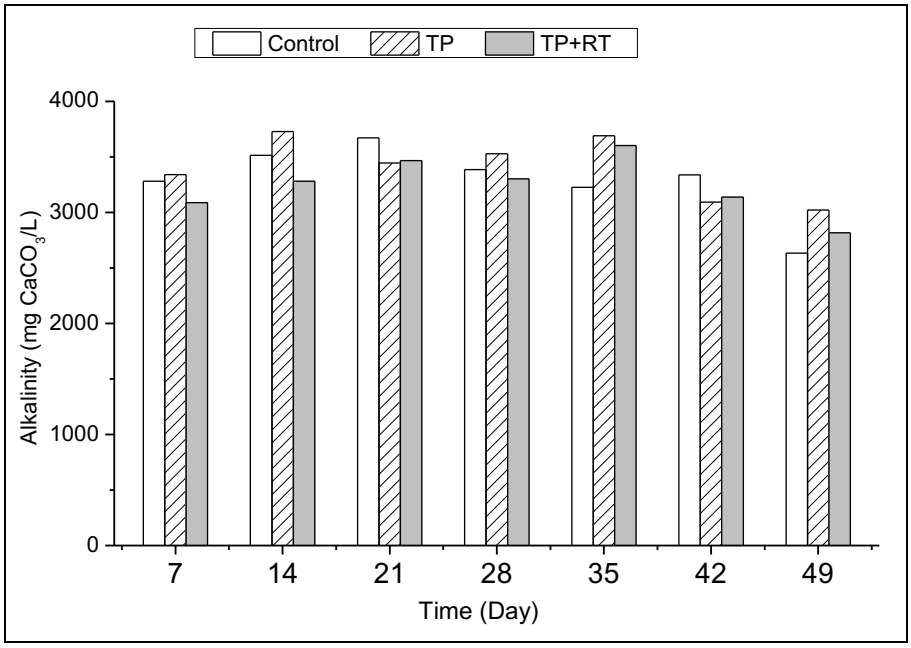


Figure 7.4 Alkalinity of weekly digestate samples from digester 1 (Control), digester 2 (TP) and digester 3 (TP+RT).

7.1.2 COD mass balance for digesters

On top of the solid removals and COD removals, COD mass balance was used to demonstrate the organic matter (tCOD) mass distribution after the digestion. As shown in Figure 7.5, based on the same organic loading (same volume of primary sludge), the COD converted to biogas, accumulated in the sludge and withdrawn from the digester via effluent (wasted sludge) were shown in the stack columns. Compared to the control digester D1 (Figure 7.5a), digester D2 (Figure 7.5b) and D3 (Figure 7.5c) had higher conversation of COD to biogas, which is consistent with the methane yield data shown in Figure 7.1. On the other hand, digester D2 had least organic matter accumulated in the digester and less COD mass in the effluent (Figure 7.5b) compared to digester D1 and D3; while digester D3 had the highest organic matter accumulation in the digestate. These observations indicated that thermal pre-treatment helped to improve the organic matter conversion to biogas, which could reduce the organic matter content in the effluent (wasted sludge). However, recuperative thickening resulted in more accumulation of COD in the digester (Figure 7.5c). It is the consequence of returning thickened sludge to the digester, therefore, more organic matter was returned to the digester. These results indicated that thermal pre-treatment would benefit the conversion of organic matter to biogas leading less organic matter residue in the digestated and wasted sludge. However, additional recuperative thickening was not in favour for reducing the organic accumulation in the digestate. Thermal pre-treatment had advantage on the resource generation (methane yield) and organic contaminants (COD) removal from the perspective of COD mass balance.

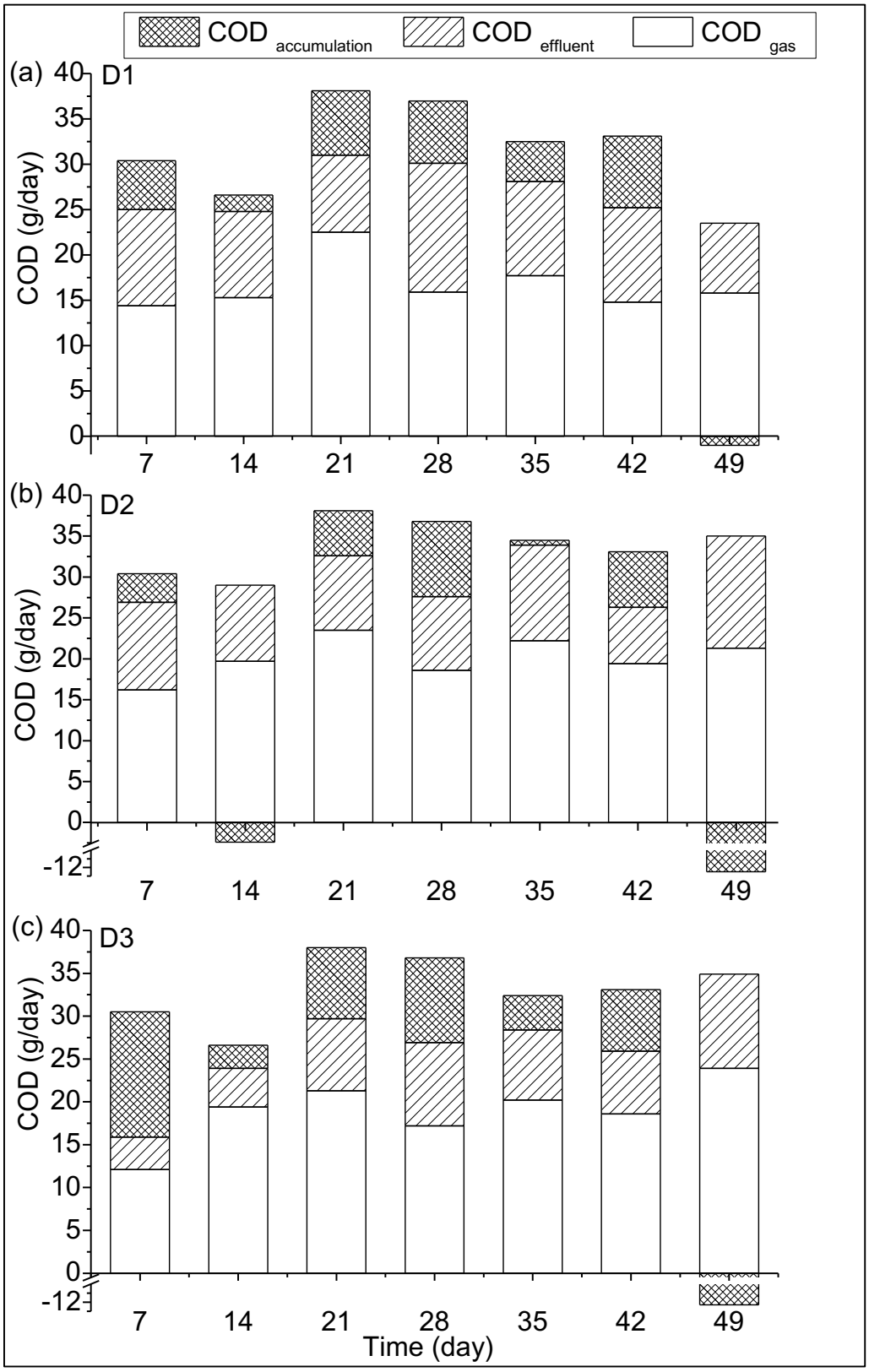


Figure 7.5 COD mass distribution by the anaerobic digester D1 (a), D2 (b) and D3 (c) during the experiment.

7.2 TrOCs occurrence in wastewater sludge

In good agreement with a previous study by Yang et al. [267], of the 40 TrOCs monitored in this study, 16 compounds were prevalently detected in all primary sludge samples (Figure 7.6). The concentrations in the aqueous and solid phase were in the range from 50 to 40,000 ng/L and from 20 to nearly 9,000 ng/g dry sludge, respectively. The occurrence of these TrOCs in primary sludge is well related to their usage in daily life. For examples, caffeine (which is a stimulant in coffee and tea) and paracetamol (which is a widely used ingredient of a pain killer) were detected at the highest concentration in the aqueous phase (40,000 and 38,000 ng/L, respectively). At the TS content of 29 g/L, it can also be inferred from Fig 3 that these TrOCs occurred mostly in the solid phase (i.e. 70 to 100% in the total mass in primary sludge). Caffeine and ibuprofen are the only two exceptions. The mass distributions of caffeine and ibuprofen in the solid phase were 24 and 41%, respectively, possibly because their hydrophilicity. These results highlight the need for specific investigation of the removal of TrOCs from the solid phase and that data from previous studies considering only the aqueous phase may not be valid in the context of anaerobic digestion.

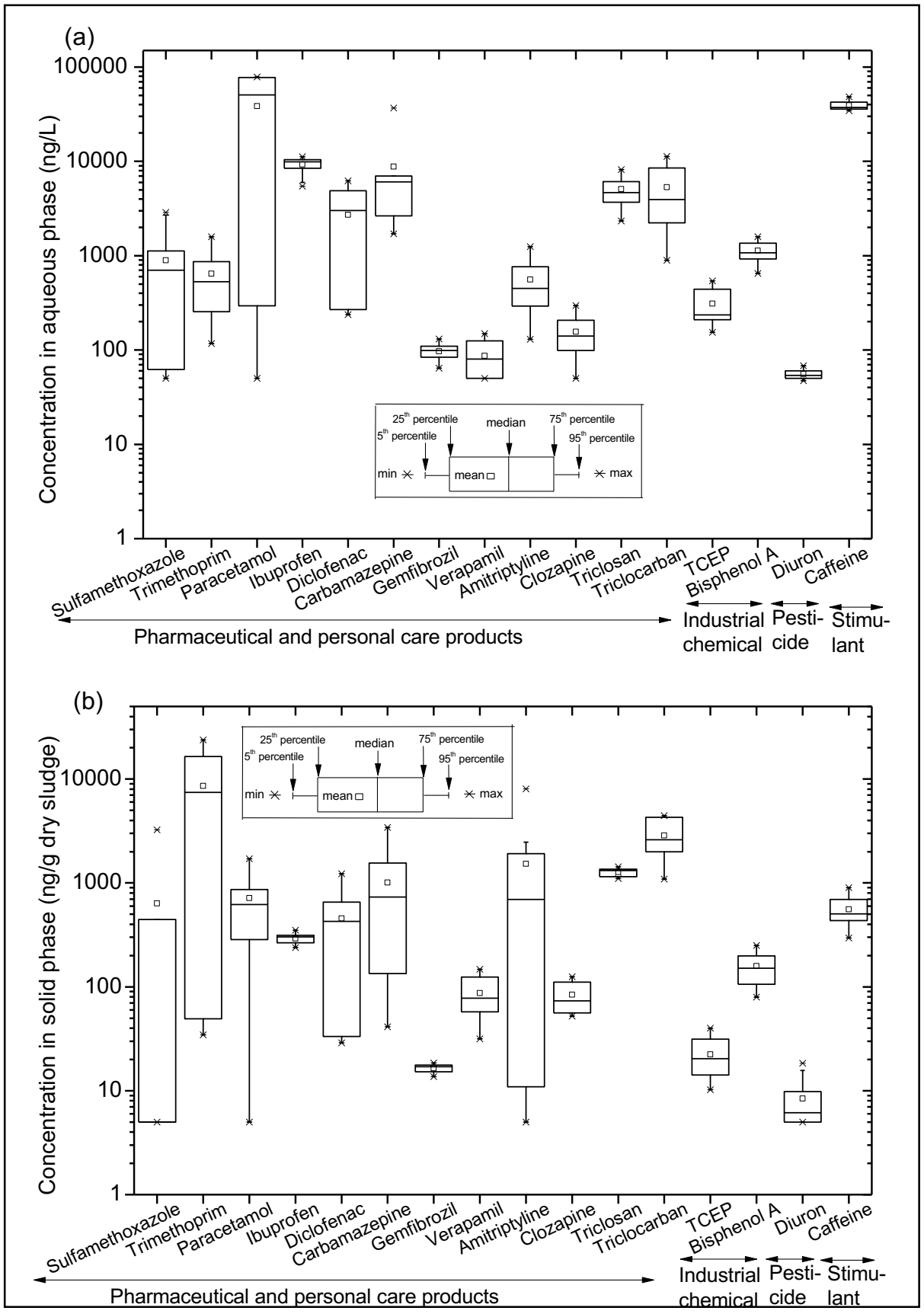


Figure 7.6 TrOC concentrations in (a) aqueous phase and (b) solid phase of primary sludge. 12 samples were taken during the experimental period.

7.3 TrOCs fate in aqueous phase and solid phase during anaerobic digestion

TrOC concentrations in the aqueous and solid phase of the feed and digestate from the three reactors are shown in Figure 7.7 and Figure 7.8, respectively. In these figures, the TrOCs were listed in the order of increasing hydrophobicity. Under all experimental conditions, caffeine and paracetamol were almost completely removed (98 – 99%) from the aqueous phase Figure 7.7. Moderately removals from the aqueous phase were observed for trimethoprim and amitriptyline especially when pre-treatment and recuperative thickening were applied together (D3). However, all other TrOCs were not significantly removed from the aqueous phase as can be observed with all three digesters (Figure 7.7). In fact, in the case of ibuprofen, gemfibrozil, and diuron, their concentrations in the aqueous phase of the digestate (after anaerobic treatment) were even higher than the corresponding values of the feed primary sludge (Figure 7.7). It is possible that the anaerobic condition could facilitate the transfer of some TrOCs from the solid to aqueous phase. This is probably because of the transfer of TrOCs from the solid phase to the aqueous phase during anaerobic digestion. It is also noteworthy from section 3.1 that most of these TrOCs are in the solid phase.

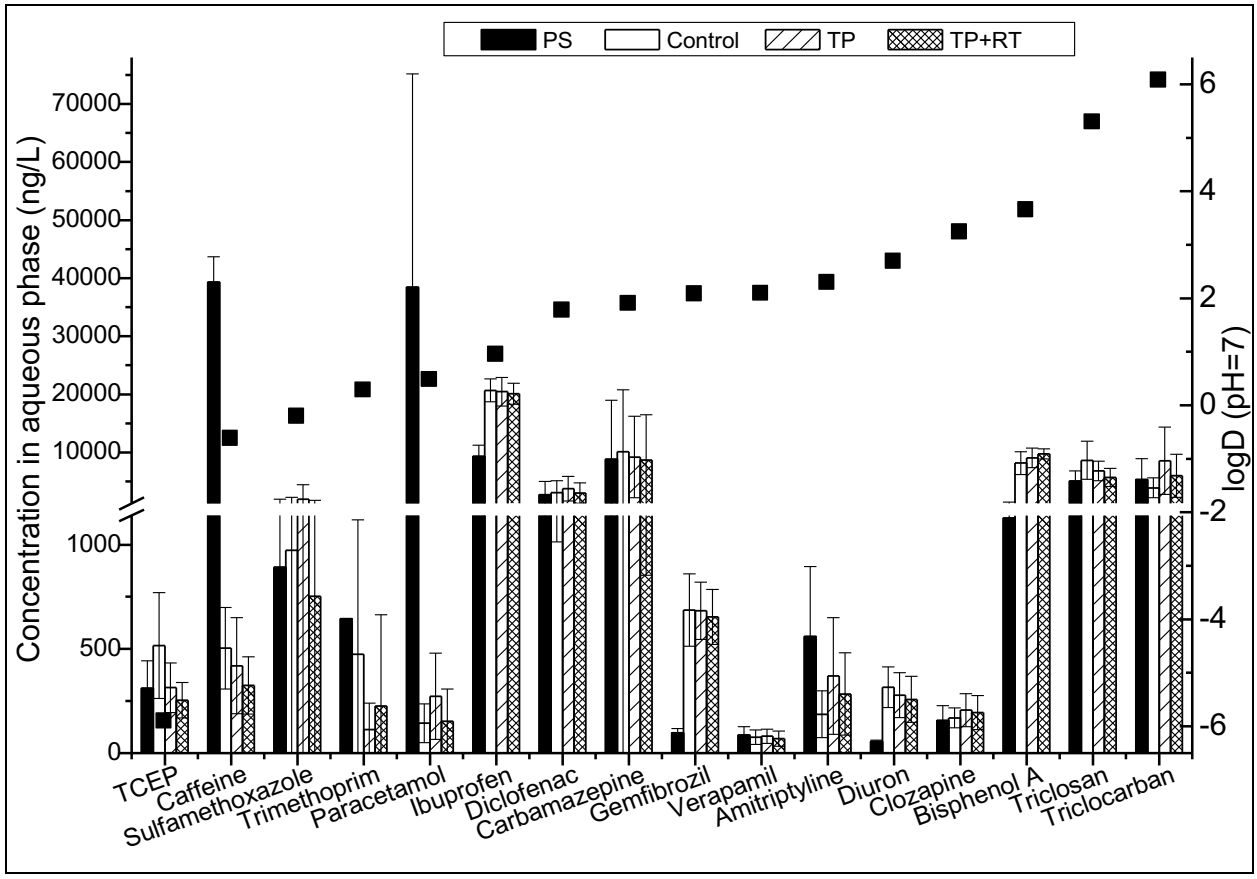


Figure 7.7 Average concentrations of TrOCs in aqueous phase of primary sludge (PS), digested sludge from digester D1 (Control), D2 (TP) and D3 (TP+RT) (mean ± standard deviation of 12 samples).

TrOC removal from the solid phase was notably higher in comparison to that from the aqueous phase. As can be seen in Figure 7.8, several hydrophilic TrOCs including caffeine, sulfamethoxazole, trimethoprim and paracetamol were well removed from the solid phase by anaerobic digestion. The hydrophilicity of compounds appears to be an important factor for their high removal from solid phase since hydrophilic compounds would easily desorb from sludge granules. However, there is no obvious evidence that Similar to the removal from aqueous phase, thermal pre-treatment or recuperative thickening could improve the removal of these TrOCs from the solid phase (Figure 7.8).

Several previous studies have also shown no discernible changes in TrOC removal after thermal pre-treatment. For example, McNamara et al. [264] reported that nonylphenol, nonylphenol diethoxylate and nonylphenol monoethoxylate were not removed from the influent by anaerobic treatment with and without thermal treatment (150 °C, 2 h). Similarly, Carballa et al. [265] also reported that thermal pre-treatment of mixed sludge by autoclaving

at 130 °C for 1 h had no impact on the removal of various pharmaceuticals, musks, and hormones by anaerobic treatment. However, it is noteworthy that these previous studies focused on the anaerobic treatment of wastewater and only consider the aqueous phase. Thus, their results cannot readily correlate to the anaerobic digestion of wastewater sludge. As discuss above, during anaerobic digestion of sludge, the transfer of TrOCs between the aqueous and solid phase can influence the overall removal efficiency. Thus, it is important to conduct a mass balance to elucidate the contribution of biodegradation and the fate of TrOCs in the aqueous and solid phase.

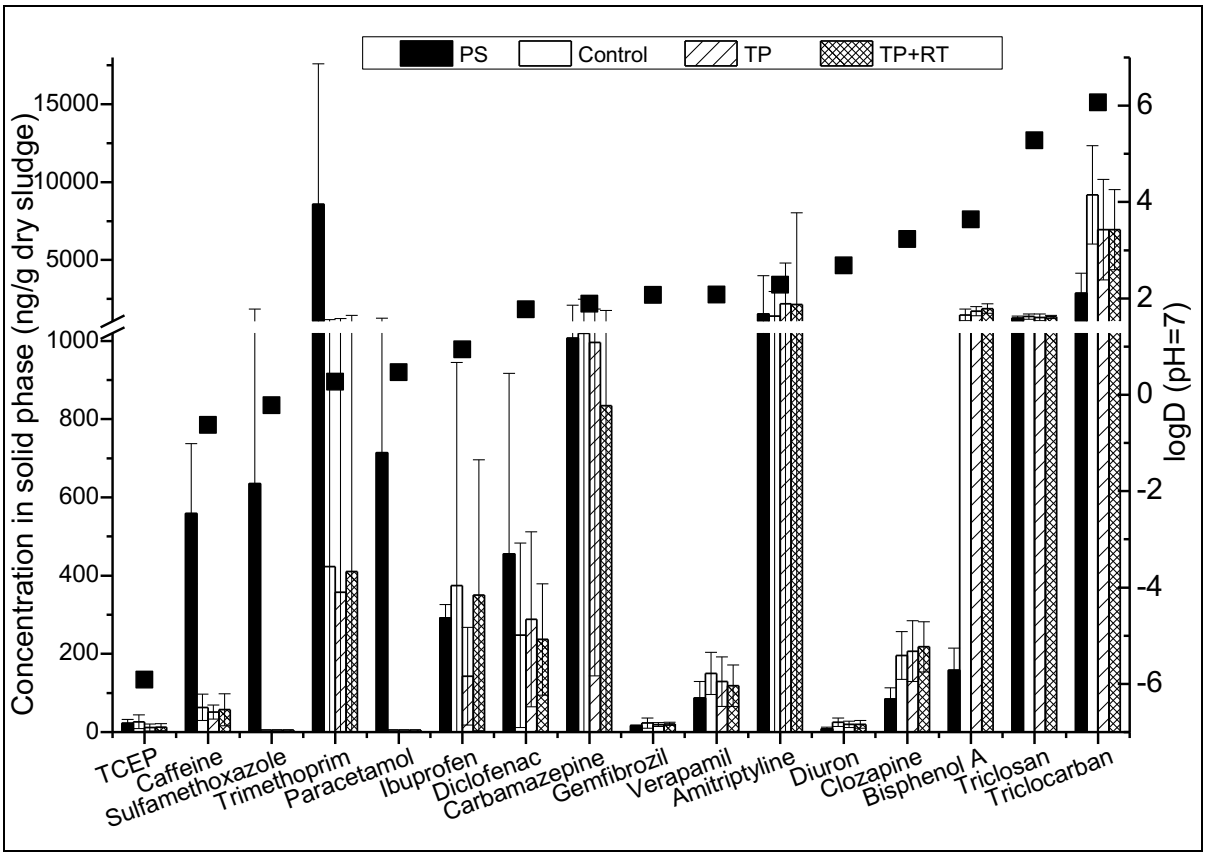


Figure 7.8 Average concentrations of TrOCs in solid phase of primary sludge (PS), digested sludge from digester D1 (Control), D2 (TP) and D3 (TP+RT) (mean ± standard deviation of 12 samples).

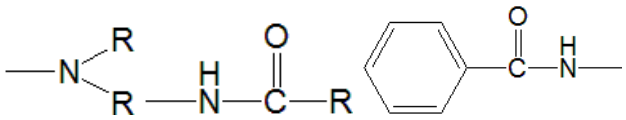
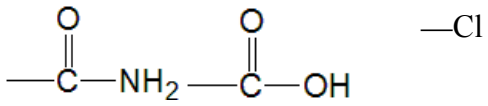
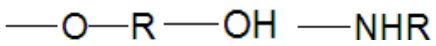
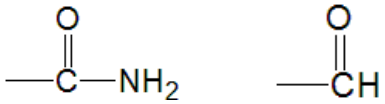
7.4 Fate of TrOCs during anaerobic digestion

Figure 7.9 shows the fate of each TrOC amongst the three possible domains namely biodegradation, partitioning to the solid phase, and partitioning in the aqueous phase. Several readily biodegradable TrOCs can be identified from Figure 7.9. They include caffeine,

sulfamethoxazole, trimethoprim and paracetamol (Figure 7.9). Likewise, four TrOCs including ibuprofen, carbamazepine, diuron and clozapine were not biodegraded under any experimental conditions in this study (Figure 7.9).

It has been established that the compound molecular structure is a major factor governing their degradability [215, 228, 255]. TrOCs with strong electron donating functional groups (Table 7.2) such as amine (caffeine, sulfamethoxazole and trimethoprim), amino (paracetamol and sulfamethoxazole), hydroxyl (paracetamol) and ether (trimethoprim) in their molecular structures are known to be readily biodegradable. On the other hand, TrOCs with strong electron withdrawing functional groups tend to be persistent to biological treatment. Examples of these electron withdrawing functional groups are carboxyl (gemfibrozil and ibuprofen), amide group (carbamazepine), chloro (diuron). Indeed, as can be seen in Figure 7.9 all TrOCs with electron withdrawing functional groups were not effectively biodegraded.

Table 7.2 Electro donating and withdrawing functional groups found in TrOCs detected in this study.

Strong electron donating functional group	Strong electron withdrawing functional group
	
	

Results from this study are consistent with several previous studies. Caffeine [61, 255], trimethoprim [61, 268] and sulfamethoxazole [61, 269] have been reported to be well removed by anaerobic digestion. By contrast, carbamazepine [61, 268, 269], diuron [228, 269] and ibuprofen [231, 268] were persistent to anaerobic digestion.

Of a particular note, enhanced biodegradation due to either thermal pre-treatment and/or recuperative thickening was observed with five TrOCs (denoted in Figure 7.9 with #). The biodegradation of triclosan and triclocarban were improved by approximately 10% due to thermal pre-treatment (Figure 7.9a and b) and further improved (by about 15%) when recuperative thickening was also applied (Figure 7.9c). Verapamil and clozapine were approximately 20% more biodedegraded when thermal pre-treatment and recuperative thickening both applied (Figure 7.9a and c). However, with thermal pre-treatment and

recuperative thickening, TCEP biodegradation increase to approximately 40% and 60% respectively.

The positive impact of thermal pre-treatment and recuperative thickening does not seem to be governed by the compound hydrophobicity. Indeed, of the 16 TrOCs in Figure 7.9, TCEP is the most hydrophilic while triclosan and triclocarban are the most hydrophobic. It appears that the removal of TrOCs with electron withdrawing functional groups (thus these TrOCs are inherently persistent to biodegradation) is likely to benefit from thermal pre-treatment and recuperative thickening. All five TrOCs discussed here have at least one electron withdrawing functional group their molecular structure.

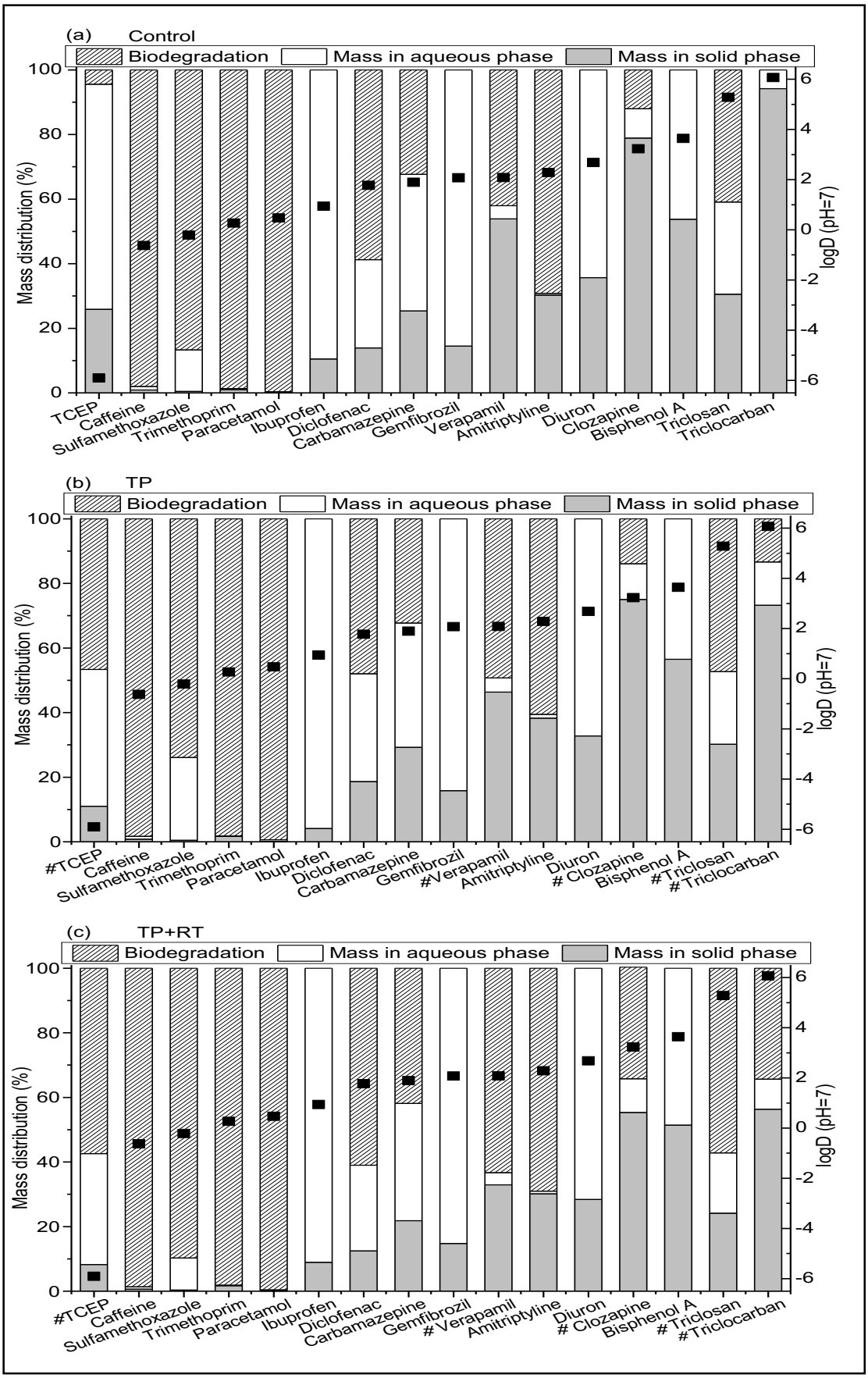


Figure 7.9 Overall fate of each compound by anaerobic digestion in digester (a) D1 (Control), (b) D2 (TP) and (c) D3 (TP+RT).

7.5 Conclusions

The effects of thermal pre-treatment and recuperative thickening on anaerobic digestion performance were examined in terms of biogas production and the removal of trace organic contaminants (TrOCs). Compared to the digester with SRT and HRT of 20 d, thermal pre-treatment and recuperative thickening resulted in approximately 15% increase in biogas production. In total, 16 TrOCs were detected in all primary sludge samples. The effects of thermal pre-treatment and recuperative thickening on TrOC removal varied significantly. Removal from the aqueous phase was insignificant for most of the 16 TrOCs detected in the primary sludge samples. Caffeine and paracetamol are the only two TrOCs with an appreciable level of removal from the aqueous phase. In comparison to the aqueous phase, TrOC removal from the solid phase was considerably higher. Through a mass balance calculation, it is revealed that thermal pre-treatment or a combination of thermal pre-treatment and recuperative thickening can enhance the biodegradation of five TrOCs, namely TCEP, verapamil, clozapine, triclosan, triclocarban by 17 - 50%.

Chapter 8 Conclusions and recommendations

8.1 Conclusion

Wastewater sludge is the semi-solid material produced from the sewage treatment of industrial or municipal wastewater. Sludge generated in wastewater treatment plants normally amounts to a small percentage (around 1%) of the volume of treated wastewater, however, the quantities of sludge produced in the modern society are increasing due to the increasing population, urbanization and upgrading of wastewater treatment plants as mandated by environmental legislation. Anaerobic digestion, as the most popular sludge treatment process in full-scale wastewater treatment plants, has caught great attention for researchers. In order to deal with increasing amount of wastewater sludge, anaerobic digesters with larger treatment capacity, enhanced sustainable resource (biogas) generation, higher organic matter reduction, better pollutants removals but lower physical footprint are highly demanded for full-scale wastewater treatment plants. Therefore, this project aims to study several approaches, like recuperative thickening and thermal pretremant, to achieve better anaerobic digester performance and pollutants removals.

In this project, three identical lab-scale continuous anaerobic digesters were used in parallel. Each digester was seeded with 20 L freshly collected digested sludge from a full-scale digester (WWTP, Wollongong, Australia), and operated under 35 °C. A peristaltic pump was used to circulate the sludge 24/7, providing sufficient mixing to the digester. Also, the pump was used to withdraw wasted digested sludge and feed primary sludge (collected from same plant) to the digester each day. The biogas production and composition of each digester were monitored daily and weekly, respectively. Sludge samples from feed sludge and digested sludge were taken regularly to monitor the sludge characteristic parameters. Additionally, sludge samples were taken for analysis of pollutants (trace organic contaminants), odour components (volatile organic sulphur compounds) and microbial community structure according to the experimental plants.

Sludge retention time (SRT) has been reported to be one of the most important factors affecting the digester performance, therefore this project firstly focused on the effect of different SRTs on the digestion and TrOC's removal. Three digesters were operated at SRT of 15 d, 20, 30 d, respectively. The results showed that,

- Biogas production, TS reduction, VS reduction, COD removals were remarkably improved by higher SRT.
- 18 of TrOCs were observed with significant occurrence, among them, paracetamol, caffeine, ibuprofen and triclosan were also found at high concentrations (>10,000 ng/L) in the aqueous phase of primary sludge.
- TrOCs with electron donating functional groups were more easily to be removed. The lack of SRT influence on TrOC removal suggests that TrOCs were not the main substrate for anaerobic digestion

Recuperative thickening is a modified anaerobic digestion process to extend SRT from HRT, which can increase SRT of the digester without decreasing the treatment capacity. This project then set out 3 experiment campaigns to study the digesters performance at different SRTs ranging from 15 d up to 60 d. The findings include,

- The increment of biogas production and system stability were observed with increased SRT.
- Recuperative thickening was effective when the digester had inadequate HRT (i.e. < 15d).
- Recuperative thickening did not enhance the organic matter destruction (removal of VS and tCOD) at a sufficiently high baseline SRT value.
- Recuperative thickening also led to improved sludge dewaterability and a reduction in total volatile organic sulphur compounds, resulting in less odorous biosolids.

Shearing can be introduced by thickening process like rotary drum or centrifuge in the full-scale operation. Thus, this project conducted 2 sets of experiments to elucidate the impact of shear force on the anaerobic digestion performance, microbial community structure and TrOC's fate when recuperative thickening was applied. Three digesters were all operated with recuperative thickening, while different shearing levels were applied to the thicken sludge during the thickening process. The results show that,

- Biogas production could be improved at medium shearing. By contrast, excessive or high shearing led to a marked decrease in biogas production, possibly due to sludge disintegration and cell lysis.
- Microbial analysis showed that medium shearing increased the evenness and diversity of the microbial community in the anaerobic digestion, which is consistent with the observed improved biogas production.

- In good agreement with the observed decrease in biogas production, the abundance of *Bacteroidales* and *Syntrophobaterales* (which are responsible for hydrolysis and acetogen) decreased due to high shearing during recuperative thickening.
- Excessive shearing was observed to deteriorate the dewaterability of the digested sludge, and digester with medium shearing was observed with least odour components in the dewatered cake.
- 17 TrOCs detected in all sludge samples constantly, and compounds like caffeine and triclocarban were found in high concentration in primary sludge.
- Some compounds like caffeine, paracetamol and naproxen were well removed from aqueous phase and solid phase regardless the shearing level.
- Compared to the other shearing conditions, high shearing improved most of TrOCs biodegradation.

Another anaerobic digestion enhancement approach, thermal pretreatment was studied in terms of the digester performance improvement and TrOCs fate. Thermal pretreatment at 150 °C for 30 min was applied to primary sludge prior to feeding for experimental digesters, while control digester only feed with original primary sludge. Results showed that,

- Thermal pretreatment increased the methane yield by approximately 30%, but additional recuperative thickening did not improve the methane yield significantly.
- Thermal pretreatment or recuperative thickening barely affected the removal of TS, VS and tCOD throughout the experiment, while the removal of sCOD was enhance only when recuperative thickening applied.
- 16 TrOCs were detected in all sludge samples. Among them, caffeine, paracetamol, ibuprofen and carbamazepine were found high concentration in primary sludge.
- Hydrophilic compounds like caffeine, paracetamol, sulfamethoxazole and trimethoprim were well biodegraded in all digesters, and hydrophobic compounds like clozapine, triclosan and triclocarbon were more biodegraded when thermal pretreament or recuperative thickening were applied.
- For the compounds residue not biodegraded during the anaerobic digestion, their mass distribution in the aqueous phase and solid phase were correlated to their hydrophobicity/ hydrophilicity.

8.2 Recommendations for future research

For future research, there are a few recommendations based on current project progress:

1. Pilot experiment need to be conducted for future study in order to confirm the observation from the lab-scale digesters. Recuperative thickening and thermal pretreatment need to be examined in pilot digesters in order to conduct the feasibility study for full-scale digesters.

2. As recuperative thickening and thermal pretreatment have been shown to enhance the biogas production (sustainable resources generation) in the lab-scale digesters, energy balance calculations should be carried out in both lab-scale and pilot digesters to understand the energy recovery from the modified anaerobic digestion.

3. Co-digestion with other substrate like food wastes, beverage waste, dairy wastes, should be studied for different scales of digesters, which will help to enlarge the spectrum of solid wastes that wastewater treatment plants can handle.

4. TrOCs, as emerging pollutants, should be monitored in the effluent and solid waste from full-scale wastewater treatment plant in order to understand and control the potential risk to ecosystem and human health.

5. The metabolites arising from degradation by anaerobic digestion need to be analysed to understand the degradation pathways; and toxicity of final digestate should be assessed.

6. Microbial community selection for anaerobic digestion will be of great importance for full-scale digester in terms of enhanced digester performance and stability.

Publications related to this project

1. Shufan Yang, Faisal I. Hai, William E. Price, James McDonald, Stuart J. Khan, Long D. Nghiem. 2016. Occurrence of trace organic contaminants in wastewater sludge and their removals by anaerobic digestion. *Bioresource Technology*, v. 210, p. 153-159.
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3. Shufan Yang, Faisal I. Hai, William E. Price, James McDonald, Stuart J. Khan, Long D. Nghiem. 2017. The fate of trace organic contaminants in sewage sludge during recuperative thickening anaerobic digestion. *Bioresource Technology*. Accepted 4 Feb 2017. http://dx.doi.org/10.1016/j.biortech.2017.02.020.
4. Shufan Yang, Faisal I. Hai, William E. Price, James McDonald, Stuart J. Khan, Long D. Nghiem. Under review. Effects of thermal pre-treatment and recuperative thickening on the fate of trace organic contaminants during anaerobic digestion of sewage sludge. *International Biodeterioration & Biodegradation*. Submitted 25 March 2017.
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